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## CAB OF CANAVALIA CAUSED BY ELSINOE CANAVALIAE<sup>1</sup>

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### INTRODUCTION

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In connection with investigations of diseases caused by members of the form genus *Sphaceloma* De Bary (4)<sup>2</sup> and the ascomycetous genus *Plectodiscella* Woronichin (44, p. 232), the writer has had occasion to make a study of a little-known disease of Canavalia (*Canavalia* DC.), including the classification of its causal fungus. The art examination of certain other myriangia referred to above. As supplementing those al which are mentioned or cited in this account d are here given for reference in connection is but primarily because of their direct bearing on the Canavalia disease and on a destructive disease of Lima bean (*Phaseolus lunatus macrocarpus* Benth.) now being investigated (19). The work is based upon material contained in the Mycological Collections of the Bureau of Plant Industry and in the United States National Herbarium.

### SPECIES OF CANAVALIA AFFECTED

The disease of Canavalia here discussed has been reported as affecting two species of this genus, *C. gladiata* (Jacq.) DC. of Asiatic origin (28) and *C. ensiformis* (L.) DC. which "it is practically certain \* \* \* is native to America." According to Piper (28) the former species is commonly known as sword or saber bean and the latter as jack or horse bean, and the two have been greatly confused. Raciborski (29, t. 1, p. 14),<sup>3</sup> who, so far as known, discovered as well as first reported the disease about 30 years ago, noted it as affecting the sword bean, that is, *C. gladiata*.

In the four reports of the disease from Ceylon that have been made it is listed once as affecting "sword bean," no scientific name being given (25), and once as affecting "*Canavalia ensiformis*" with no mention of a common name (26); in the two other reports one or both common names, sword and saber bean, are given and also the binomial *Canavalia ensiformis* (7, 8). Practically the same situation is found in the four Philippine reports of the disease which are available (2, 3, 31, 43). Thus there exists here the confusion referred to by Piper. In the case of the reports of the disease from

<sup>1</sup> Received for publication July 21, 1930; issued January, 1931. This is a somewhat amplified section of a paper read before the Botanical Society of America, Mycological Section, Dec. 30, 1929. The illustrations are by J. F. Brewer.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 10.

<sup>3</sup> Buitenzorg, Java. M. Raciborski. (RACIBORSKI, M. CRYPTOGRAMAE PARASITICAE IN INSULA JAVA LECTAE EXSICCATAE. Fasc. 1, No. 9. Buitenzorg. 1899.)



Ceylon it would seem that the host concerned is *C. gladiata*. Increasing the uncertainty of the exact species of Canavalia affected in the Philippines is the information recently received from E. D. Merrill<sup>4</sup> that the "*C. ensiformis* of most Philippine authors appertains to the endemic *C. luzonica* Piper \* \* \*." It was learned from the same source that "*C. gladiata*" and "*C. ensiformis*" are "not common and \* \* \* never spontaneous in the Philippines," where the latter or West Indian species was apparently "introduced \* \* \* after the American occupation." Thus, although *C. ensiformis* has been listed as susceptible to the Canavalia disease here discussed, it is not definitely known at present whether this species is actually affected. The material in the United States National Herbarium referred to consists of the terminal part of a main stem or branch and one empty pod of Canavalia collected in the Singapore Botanic Gardens in April, 1921. This specimen, recently found by the writer to be severely affected by the disease, was labeled *C. ensiformis* when acquired, but it had later been identified as *C. gladiata*(?) by Piper. Had seeds been available it is probable that he would not have questioned his specific identification of this specimen.

## THE DISEASE

### NAME

In the small amount of literature concerning the disease under discussion there is no mention of a name by which it may be designated. On this account, and because of its similarity to the rutaceous disease commonly known as citrus scab, this legume disease is here referred to as scab of Canavalia.

### HISTORY, RANGE, AND IMPORTANCE

As already indicated, scab of Canavalia, so far as known, was discovered in Java by Raciborski about 30 years ago and has since been observed in the Philippines and Ceylon. Based upon the scabbed condition of the specimen from Singapore found by the writer in the United States National Herbarium, its range is now known to include the Malay Peninsula. Other than notations of its occurrence in Ceylon and in the Philippines and the two brief accounts by Raciborski (29, t. 1, p. 14) and Arnaud (1, p. 685-686) dealing mainly with its leaf symptoms, the disease has scarcely been investigated and little definite information is available concerning its importance. Raciborski reported that in Java it affects leaves, stems, and fruits, producing swellings that resembled those due to Exoascaceae. Baker (3, p. 159) stated that in the Philippines it caused "widespread distortion and death of both leaves and stems." This disease is probably one of the most important of those affecting Canavalia species susceptible to its attack.

### SYMPTOMS

Raciborski stated that on fresh leaves (29, t. 2, p. 3) the lesions measured 2 to 12 mm. in diameter by about 1 to 3 mm. in thickness. Their general appearance as further described by him is represented by the Raciborski specimen already cited and by a specimen from

<sup>4</sup> Letter dated March 10, 1930.

the Philippines collected by Baker<sup>5</sup> in 1913, illustrations of which are shown in Plate 1, A-C. These illustrations show, in addition, the shot-hole effect apparently resulting from the falling out of the lesions. Lesions not noticeably swollen are visible on the midrib and veins of one of the leaves. (Pl. 1, C.)

The leaf lesions on the herbarium specimens from Singapore are generally brighter in color and larger than those described by Raciborski. These lesions are here bone brown<sup>6</sup> at the center, varying to Mars brown toward the outside. The scattering, more or less circular pod lesions, which are generally flat but occasionally slightly raised or depressed, show practically the same coloration as the leaf lesions. The numerous stem lesions, which are circular, elliptical, or occasionally elongated, are paler, that is, cinnamon brown, somewhat grayed. The circular leaf lesions are about 6 mm. across; others following the veins attain a length of 2 cm. and a width of 4 mm. The larger pod lesions are about 3 mm. in diameter, while the stem lesions may extend for a distance of 2 cm. along the shoot, enveloping more than half its circumference. The histologic structure of the galls (pl. 1, D) as studied by Raciborski, Arnaud, and the writer appears to be homologous with that of the leaf lesions of citrus scab as recently studied by Cunningham (9). In other words, the 2-layered development reported by Arnaud undoubtedly corresponds to what in leaf lesions of citrus scab Cunningham designates as the formation of a "phellogen in the spongy parenchyma \* \* \* as a result of" whose activity " \* \* \* a definite phellem is laid down, thus completely isolating the portion invaded" by the causal fungus, *Sphaceloma fawcettii* Jenkins. The gall formation was here due to an increase in the number as well as in the size of the cells in the spongy parenchyma.

## THE PATHOGENE

### MORPHOLOGY

#### HYPHAE

The hyphae of the genus *Elsinoe*, in which is classified the causal fungus of scab of *Canavalia*, are described (29, t. 1, p. 14) as intercellular and as forming a thick, colorless or light-gray pseudoparenchyma between the epidermis and the mesophyll, which remains covered by the epidermis for a considerable period. Those of the pathogene are said (29, t. 1, p. 14) to be barrel-shaped and the stromatic layer to range from 10 $\mu$  to 38 $\mu$  in thickness. In its orientation Arnaud (1, p. 685 and pl. 3, C) observed that the stromatic layer is found just outside the diseased tissue here termed the "phellem" and that it also grows into the outer cells of this tissue as well as beneath the disrupted epidermis.

#### IMPERFECT STAGE

Neither Raciborski nor Arnaud reported a conidial stage for the pathogene of scab of *Canavalia*, but such a stage doubtless exists. In fact, small, spherical, hyaline bodies, interpreted as microconidia

<sup>5</sup> Mount Maquiling, near Los Baños, Province Laguna Luzon, Philippines, 1913, C. F. Baker. (BAKER, C. F. FUNGI MALAYANA Century I, No. 26.) Specimen in Mycological Collections of the Bureau of Plant Industry.

<sup>6</sup> The color readings in this account were made by J. Marion Shull and are based on the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE, 43 p. illus., Washington, D. C. 1912.

of the *Elsinoe*, were seen by the writer on some of the material examined. This consists of the specimens from Java, Singapore, and the Philippines already cited, as well as an additional Philippine specimen collected by Baker <sup>7</sup> in 1914. The microconidia were similar to those recently reported by the writer (17) for *Sphaceloma symphoricarpi* Barrus and Horsfall, in which form genus, created by De Bary (4), the conidial stage of this fungus is certainly to be classified. Acervuli are probably present on the material examined, but a critical search has not been made for them. The microconidia seen were possibly produced as sprout conidia from ascospores. In Plate 3, C, *a*, are shown similar although somewhat larger structures interpreted as microconidia of *Uleomyces sanguineus* (Speg.) Syd.<sup>8</sup> (38, p. 219), which is the type of the genus *Uleomyces* P. Henn. (12, p. 107), referred to later. These indicate a similar conidial stage for this related species not hitherto noted.

#### PERFECT STAGE

Raciborski (29, t 1, p. 14) and Arnaud (1, p. 685, and pl. 3, C) reported that the single layer or groups of asci in the causal fungus of scab of *Canavalia* develop in the stromatic layer already mentioned. (Pl. 1, D.) Raciborski gave the measurements of the globular asci and of the transversely septate hyaline spores, eight in an ascus, as  $16\mu$ - $19\mu$  by  $20\mu$ - $22\mu$  and  $9\mu$ - $12\mu$  by  $2.8\mu$ - $3.5\mu$ , respectively. Upon discovering that the spores in the Javanese material collected by Raciborski <sup>9</sup> were not colored and muriform as in *Uleomyces*, where, as will later be more fully explained, Arnaud classified the fungus, he attributed this condition to the immaturity of the specimen. Longitudinal septation might be delayed as in *Uleomyces sanguineus*, he explained.

On the specimen from Singapore there were seen both asci and spores judged to be more mature than those described by Raciborski or than those seen by Arnaud. The largest asci, which were here clavate and furnished with a short stipe, attained a size of  $24\mu$ - $30\mu$ , while the largest spores, some of them still within the ascus, measured  $28\mu$  by  $9\mu$ . The more mature spores ranged from hyaline to yellowish or greenish yellow. The transverse walls were well defined, but longitudinal septation, which Arnaud stated might be delayed, was not seen. In the thickened apical region the walls of a number of the asci were faintly stained, apparently from the coloring matter in the surrounding matrix. Petch (27, p. 62-63) has made a similar observation in the case of *Myriangium duriae* Mont. and Berk. (6, p. 73), the type of the genus *Myriangium* Mont. and Berk. (6, p. 72-73), on which was founded the order Myriangiales Starbäck <sup>10</sup> (35), referred to in the following section. Here, not only the ascus wall but also the spores

<sup>7</sup> Los Baños, Province Laguna, Luzon, Philippines, 1914, C. F. Baker. (SYDOW, FUNGI EXOTICI KASIMPATI, Fasc. 9, No. 420.) In Mycological Collections of the Bureau of Plant Industry.

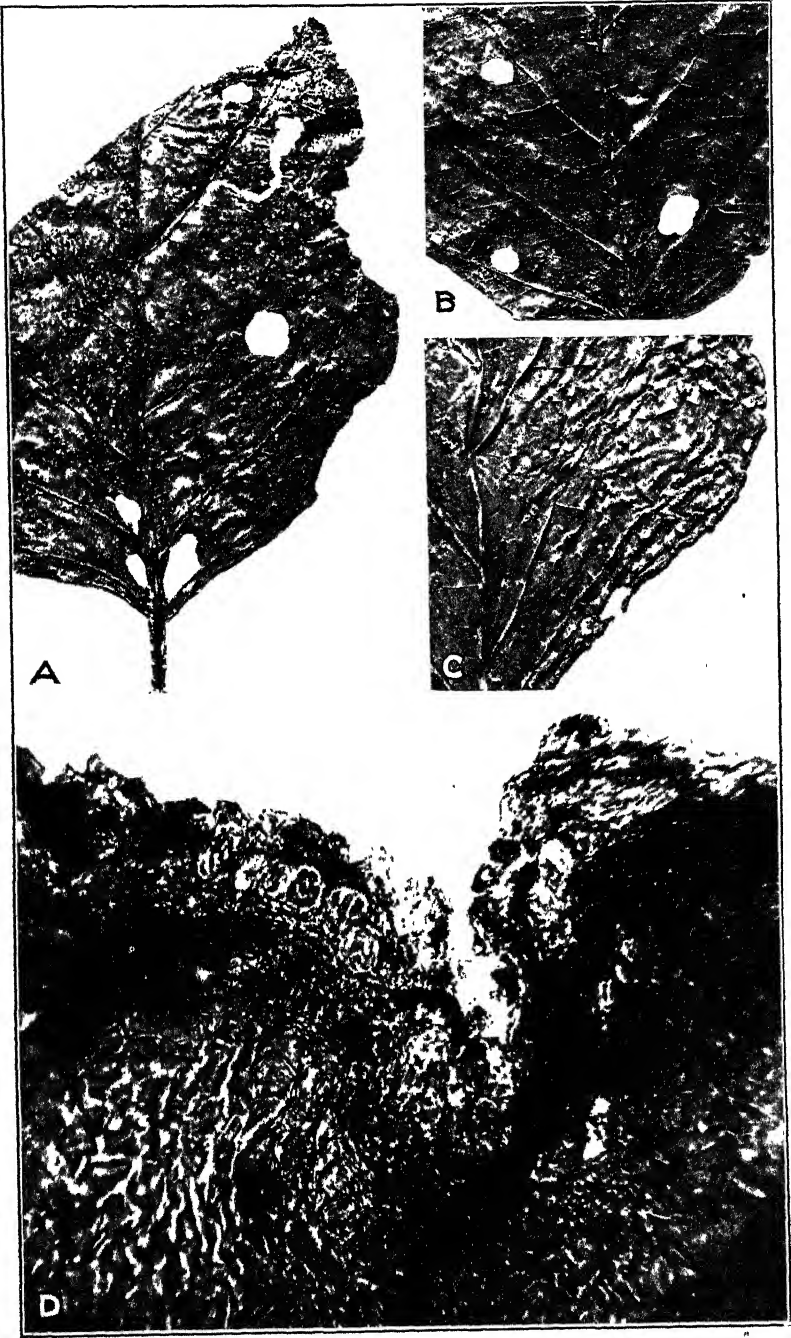
<sup>8</sup> Synonyms: *Uleomyces parasiticus* P. Henn. (12, p. 107), *Cookella parasitica* (P. Henn.) P. Henn. (13, p. 275), and others referred to later.

<sup>9</sup> Op. cit. (See footnote 3.)

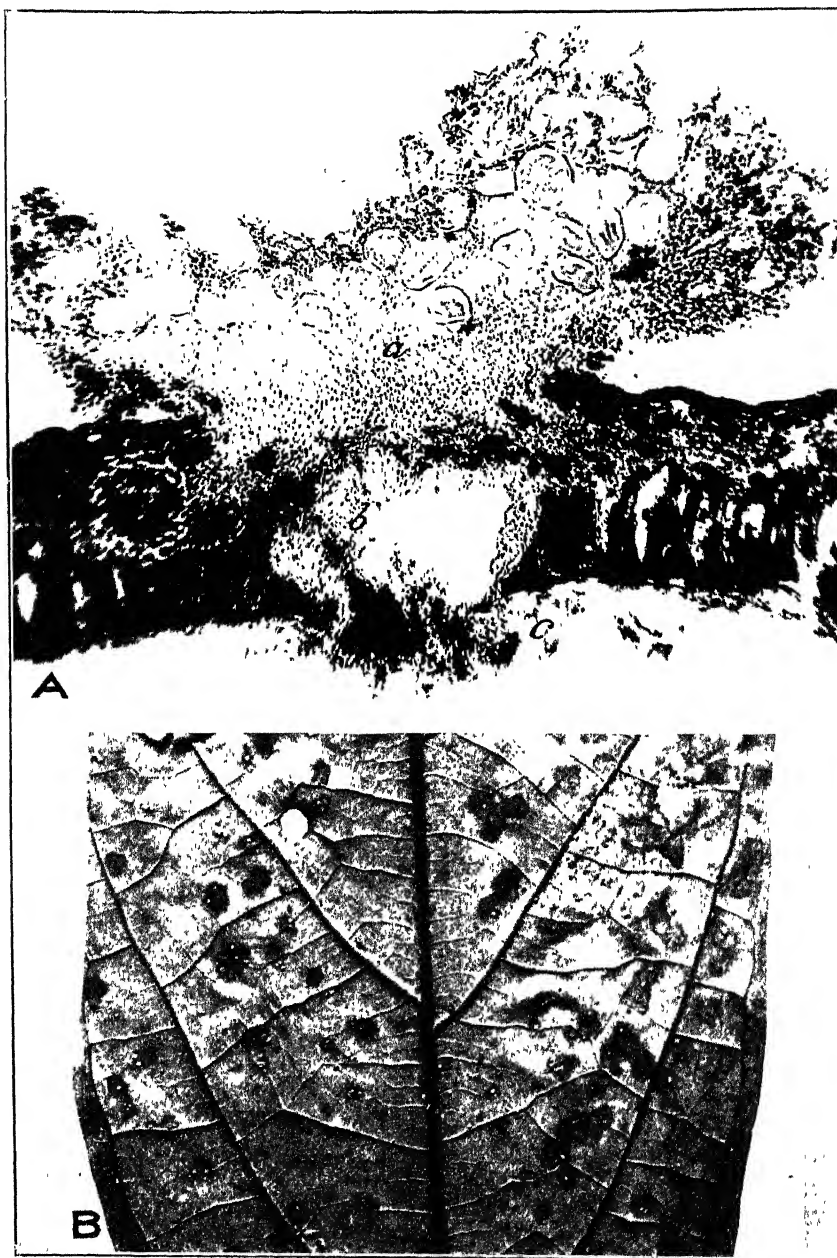
<sup>10</sup> Synonym: Myriangiacei Nylander (23, p. 139).

#### EXPLANATORY LEGEND FOR PLATE I

Leaves of *Canavalia* infected by *Elsinoe canavaliae*. Upper (B) and lower (A and C) leaf surfaces, and gall (D) in median cross section, showing stromatic fertile layer, shot-hole effect, and, particularly in C, lesions on midrib and veins. A-C,  $\times 1$ ; D,  $\times 380$ . Material for A and B collected by C. F. Baker, Mount Maquiling, near Los Baños, Province Laguna, Philippines, 1913 (C. F. Baker, Fungi Malayani Cent. 1, No. 20); specimen in Mycological Collections of the Bureau of Plant Industry. Material for C and D collected by M. Raciborski, Buitenzord, Java, April, 1900 (M. Raciborski, Cryptogamiae Parasiticae in insula Java lectae exsiccatae Fasc. 1, No. 9, Buitenzord, 1898).



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within the ascus were stained brown, as it seemed, from the "adherent \* \* \* decaying" stroma. So far as the writer knows, only hyaline spores had previously been reported for this species. It may be mentioned here that only the supposed lack of colored spores in *Myriangium* prevented Hennings (14, p. 354) in 1901 from transferring to this genus his *Uleomyces parasiticus*, i. e., *U. sanguineus*, at the time he recognized its identity with *Phymatosphaeria sanguinea* Speg.<sup>11</sup> (34, p. 57). This would have made the genus *Phymatosphaeria* Speg. (34, p. 57) a synonym under the older genus *Myriangium*. In the classification of Theissen (40, p. 312) and Theissen and Sydow (41, p. 439), the genera *Uleomyces* and *Myriangium* are separated on the basis of the distribution of the asci in the ascoma.

#### NAME, HISTORY, AND CLASSIFICATION

Scab of Canavalia is caused by *Elsinoe canavaliae* Rac. (29, t. 1, p. 14). This fungus is the type of the genus *Elsinoe* Rac. (29, t. 1, p. 14). Raciborski classified the genus and therefore the species in the family Exoascaceae near *Magnusiella*. In 1909, in connection with his revision of the order Myriangiales, or family Myriangiacei, as he treated the group, Von Höhnelt (16, p. 349-376) removed this genus and species from the Exoascaceae, raised to generic rank *Myriangina*,<sup>12</sup> formerly classified as a subgenus of *Myriangium* by its author, Hennings (15, p. 55), and then erected for them the new family *Elsinoëen*. This new family he considered to be of doubtful systematic position. *Myriangina mirabile* he stated might be regarded as an ingrown form of the Plectascales, while in his opinion the genus *Elsinoe* afforded a transitional form not theretofore reported in that order. He concluded that the relationships of the new family might be with the Plectodiscales or the Protodiscales, but that the final solution of its classification rested upon transitional forms as yet undiscovered.

*Myriangina mirabile* is based upon material collected in Brazil in 1901,<sup>13</sup> which has been examined by the writer. For comparison with *Elsinoe canavaliae*, illustrations from material found in Brazil on leaves of a species of Lauraceae,<sup>14</sup> (on which host the fungus was originally described) and on leaves of an undetermined host<sup>15</sup> are here shown in Plate 2 and in Plate 4, D-G. Plates 2, B, and 4, D, show the general appearance of the ascomata in this species. The shot-hole effect in the leaf shown in Plate 4, D, resembles that here illustrated for scab of Canavalia. (Pl. 1, A-C.) In one of the sections similar to that shown in Plate 2, A, asci were scattered about in the stroma within the leaf tissue (pl. 2, A, b), as well as in that composing the superficial ascoma. It will be noted that in this species the ascoma may protrude

<sup>11</sup> Synonym: *Ascomycetella sanguinea* (Speg.) Sacc. (33, p. 847).

<sup>12</sup> Synonym: *Dictyomollisia* Rehm, typified by *D. albidogranulata* Rehm (39, p. 403).

<sup>13</sup> Sao Paulo, Brazil, April, 1901, A. Puttemans. (PUTTEMANS, A., FUNGI S. PAULENSIS No. 176.) Specimen in United States National Herbarium (cited in 15, p. 55).

<sup>14</sup> Sao Paulo, Brazil, 1901, F. von Höhnelt. (REHM, H., ASCOMYCETES. Fasc. 33, No. 1704) (30, p. 208).

<sup>15</sup> Sao Leopoldo, Brazil, 1908, F. Theissen. (THEISSEN, F., DECADES FUNGORUM BRASILIENSIS. Century 1, No. 75, labeled *Myriangium* (*Myriangina*) *mirabile* P. Henn.) (42, p. 403.)

#### EXPLANATORY LEGEND FOR PLATE 2

*Myriangina mirabile* on leaf of Lauraceae: A, Ascomata in median cross section; a, ascomata protruding from lower leaf surface; b, ascomata within leaf tissue; c, remnants of ascomata protruding from upper leaf surface. X 380. B, Ascomata in situ on lower leaf surface. X 1. Material collected by F. Theissen, Sao Leopoldo, Brazil, 1908 (Theissen, Decades Fungorum Brasiliensis, Cent. 1, No. 75). (39, p. 403)

on either surface of the leaf. (Pl. 2, A, a and c.) The scantiness of the stroma in *Elsinoe canaraliae*, as compared to its abundance in *Myriangina mirabile*, was held by Von Höhnelt (16, p. 373) to account for the asci being smaller and less numerous in the former species than in the latter. The muriform spores of *M. mirabile* may be seen in Plates 2, A, and 4, F, while in Plate 4, G, is shown the 2-layered ascus apparently not previously reported for this genus or species. Here the "primary membrane" (pl. 4, G, a) also interpreted by Millardet (22, p. 15, pl. 3, fig. 29, b) for *Myriangium duriaei*, has become separated from what by the same author is termed the "secondary layer" (pl. 4, G, b) of the ascus in *Myriangium*. With somewhat different explanations the 2-layered ascus has been reported and illustrated by Miles (21, p. 78-79, fig. 1, a) for *M. tuberculans* Miles, by Peteh (27, p. 61-63, pl. 2, figs. 14 and 20) for *M. duriaei*, and by Stevens and Weedon (36, pl. 19, fig. 9 and pl. 20, fig. 15) for the two myriangoid species *Kusanoopsis guianensis* and *Myrianginella tapirae*, of which they are the authors. Stevens and Weedon remark upon the rarity of the double-walled ascus in the ascomycetes in this connection, citing De Bary's (5, p. 93-96, figs. 46 and 47) account of the homologous structure in the ascus in *Sphaeria scirpi* and *Pleospora herbarum*. The conidial stage of *Myriangina mirabile*, not hitherto reported, is apparently similar to that of *Elsinoe canaraliae*. Microconidia of this species, sprouted from ascospores still inclosed within the secondary layer of the ascus, are here shown in Plate 4, G. Other conidia were seen which may belong to this fungus. These were hyaline or reddish brown, minute and spherical or somewhat larger and elongate, resembling those illustrated by the writer<sup>16</sup> for *Sphaeceloma fawcettii* Jenkins.

The two genera *Elsinoe* and *Myriangina* are certainly to be classified in the Myriangiales, as the group was recognized by Theissen (40, p. 311) in 1916, and where they were placed by Theissen and Sydow (41, p. 433-447) in their arrangement of the order published the year following. These authors erected for the *Elsinoeae*, as they termed Von Höhnelt's family *Elsinoeen*, and the family *Plectodiscelleae* Woronichin (44, p. 232) the suborder *Protomyriangieae*. The other suborder in the Myriangiales as considered by them is that of *Eumyriangieae*, treated by its author Theissen (40, p. 312) as a subfamily in this order. Both Theissen (40, p. 312) and Theissen and Sydow (41, p. 439) placed the genera *Myriangium* and *Uleomyces* in this subfamily or suborder, separating them from each other as already indicated. Theissen and Sydow (41, p. 437) state that in the *Protomyriangieae* the ascoma is intramatrical and not definitely limited in extent, while in the *Eumyriangieae* it is definitely formed and erumpent with a differentiated surface.

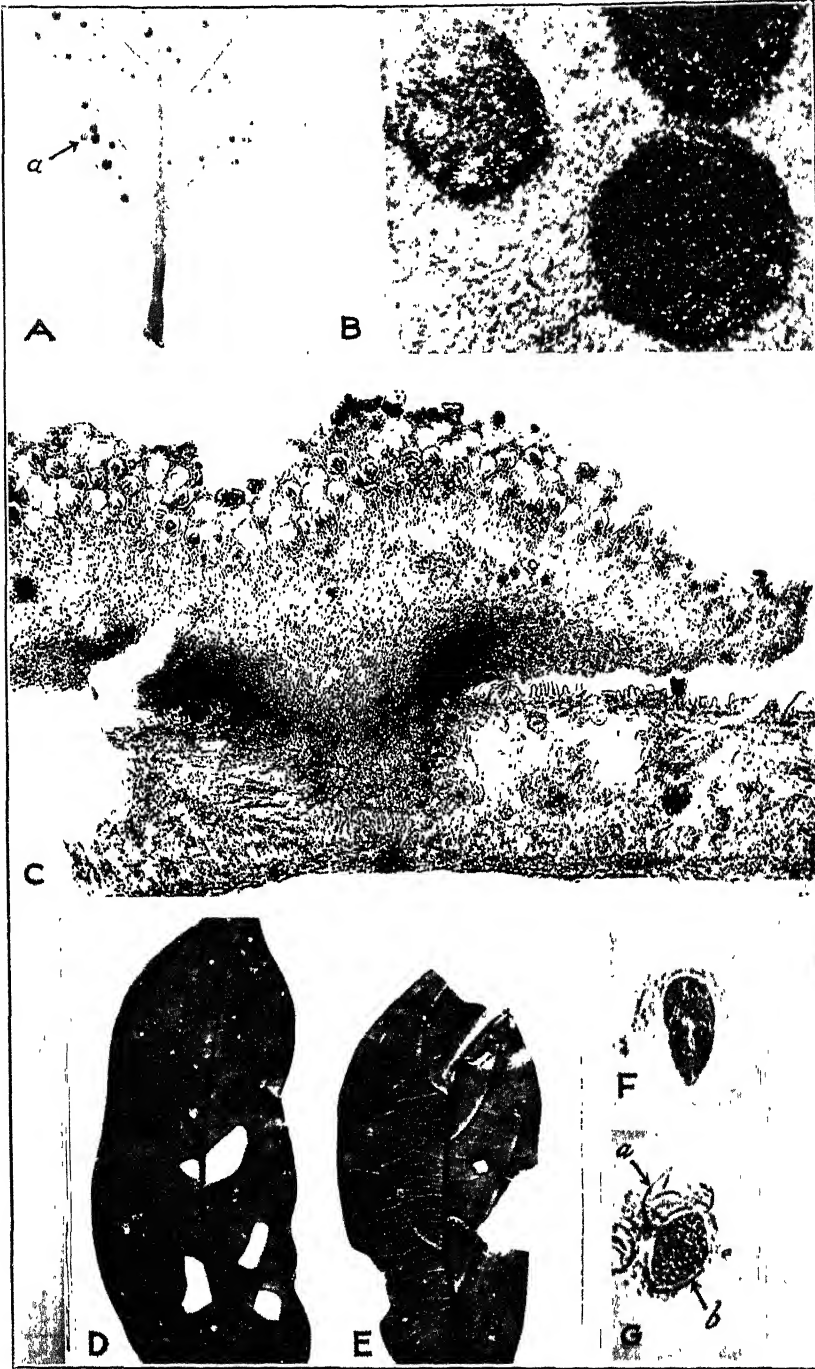
In Arnaud's (1, pp. 672-706) more recent revision of the Myriangiales the order comprises two families, the Myriangiaceae with the two tribes Cookellées<sup>17</sup> and Myriangiées, and the Saccardinulacées. As here indicated,<sup>17</sup> the *Elsinoeen* are made by Arnaud (1, p. 679) a synonym of his tribe Cookellées. In this tribe he (1, p. 676) includes the genus *Uleomyces* and one other, *Cookella*, making *Elsinoe*, *Plecto-*

<sup>16</sup> JENKINS, A. E. DEVELOPMENT OF THE CITRUS-SCAB ORGANISM, *SPHAECILOMA FAWCETTII*. Unpublished.  
<sup>17</sup> Synonyms: Cookellacées Von Höhnelt pro parte, Saccardinulacées Von Höhnelt, *Elsinoeae* Von Höhnelt, and *Plectodiscellées* Woronichin (1, p. 681)



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discella, Myriangina, and Dictyomollisia synonyms of *Uleomyces* and definitely transferring *Elsinoe canavaliae* to that genus (1, p. 685). The two species representing the monogeneric family Plectodiscelleae are also transferred. Formerly known as *Plectodiscella piri* Woronichin and *P. veneta* (Sacc.) Burkholder, they thus became *Uleomyces piri* (Woronichin) Arnaud and *U. veneta* (Burkholder) Arnaud. The first causes an important disease of apple and pear in Europe, not known to occur in this country (18, 20, 24, 44), and the second the widespread anthracnose of brambles (*Rubus*).

Although *Elsinoe* has been relegated to synonymy under *Uleomyces* and the type definitely transferred to the latter genus, it has seemed desirable here to continue to refer to both genus and species by their original names. When more data are available it is planned to discuss further the synonymy of these and the other closely related genera here mentioned and their type species. The asci and spores in the type species of the genus *Uleomyces* (pl. 3, C, b, and G) are considerably larger than in that of *Elsinoe*, measuring  $60\mu$ – $100\mu$  by  $30\mu$ – $50\mu$ , and  $22\mu$ – $32\mu$  by  $11\mu$ , respectively (12, p. 107), and are described by Spegazzini (34, p. 57) as hyaline and by Hennings (12, p. 107) as brown or deep wine color. That the latter color may be due to a staining from the bright coloration of the stroma is indicated by an observation recorded in the legend of Plate 3.

The families Myriangiaceae and Saccardinulaceae, as well as the two tribes of the former family, are separated by Arnaud (1, p. 678) on the same basis, namely, pathogenicity and characteristics of the ascoma. As he states in his key (1, p. 678), the Saccardinulaceae are pathogenic on phanerogams and the Myriangiaceae on other fungi or on insects. Previously in this account, or in the following section, it is indicated that the type species of the genus *Uleomyces* or those of the genera relegated to synonymy under this genus are actually pathogenic on phanerogamic plants, as is also apparently *Cookella microscopica*, the only species that Arnaud includes in the genus *Cookella*. The type species of the genus *Myriangium* was originally reported by Montagne and Berkeley (6, p. 73) on the phanerogamic host *Morus albus*, and Millardet (22, p. 13) found the species to be

#### EXPLANATORY LEGEND FOR PLATE 3

*Uleomyces sanguineus* and *Parmularia styracis* on leaves of *Styrax*.

- A.—General appearance of fructifications, most of which are of *Uleomyces sanguineus*.  $\times 1$   
 B, C—*Uleomyces sanguineus* in median cross section, together with microconidia (C, a) and ascospores (C, b) as originally contained in an ascus. B,  $\times 130$ ; C,  $\times 380$   
 D, E.—Enlarged entire fructifications of *Parmularia styracis* and *Uleomyces sanguineus*, respectively.  $\times 22$   
 F.—Median cross section through the *Parmularia* fruit body shown in D, the arrow indicating the central part of this rosettelike structure.  $\times 44$   
 G.—An individual ascus of *Uleomyces sanguineus* in situ in the stroma in which it was borne.  $\times 380$ . After the sections had been in mounting fluid for a time they became surrounded by a halo of purplish coloring matter diffusing from the ascomata, and the spores, which were mostly reddish brown when the mounts were made, became stained a deep purple  
 Material collected by J. Rick at São Leopoldo, Brazil, in 1904 (Rick, Fungi Austro-Americani, Fasc. 2, No. 49). (30, p. 309)

#### EXPLANATORY LEGEND FOR PLATE 4

- A–C.—Ascomata of *Uleomyces decipiens* on lower surface of leaf of *Pasania glabra*. A,  $\times 1$ ; B, enlargement of A, a,  $\times 22$ ; C, median cross section through one of the ascomata illustrated in B, showing its direct attachment to the leaf,  $\times$  about 100. Material collected by T. Yoshinaga, Japan, 1921 (Sydow, Fungi Exotici Exsiccati, Fasc. 2, No. 513). (37)  
 D–G.—Upper (D) and lower (E) surface of leaf of undetermined host attacked by *Myriangina mirabile*, showing shot-hole effect resulting from the falling out of the lesions, and the white ascomata of this fungus on the upper leaf surface.  $\times 1$ . F and G, Two asci, in one of which (G) microconidia have sprouted from the spores, giving them a granular appearance, and in which the primary membrane (a) of the ascus has become separated from the secondary layer (b).  $\times 380$ . Material collected by F. von Höhnel, Rio Paulo, Brazil, 1901 (H. Rehm, Ascomycetes, Fasc. 39, No. 1704). (30, p. 208)

definitely pathogenic on *Nerium oleander*. According to Arnaud, it was Zimmermann (45, p. 876) who first indicated "assez timidement" that *Myriangium duriaei* was parasitic on a scale insect. Zimmerman stated that the *Myriangium* grew within and on the surface of *Ichnopsis filiformis*, occurring on leaves of *Coffea liberica* and Elias in Java. He explained, however, that the fungus was not joined to the body of the *Ichnopsis* and that perhaps it penetrated only the scale covering of the dead insect. His illustrations suggest that, although the insect and the fungus were closely associated, the latter may have been growing directly on the phanerogamic substrata.

Assuming the direct pathogenicity on phanerogams of the fungi mentioned above, this would leave only the criterion of mycological differences, that is, characteristics of the ascoma, to distinguish the families, tribes, and, to a certain extent, even the genera composing the Myriangiales of Arnaud. Whether the differences in the fructifications are sufficient to warrant the formation of more than one family or tribe in the order is debatable. Further knowledge of the group will assist in elucidating these points.

Nylander (23) placed the family Myriangiaceae among the lichens, but it was definitely recognized as belonging to the fungi by Millardet (22) in 1870. That author stated that the affinities of the group appeared to be with the Tuberaceae and that it should receive equal ranking with this group. Spegazzini (34, p. 57), creating the family Phymatosphaeriaceae, typified by *Phymatosphaeria sanguinea*, i. e., *Uleomyces sanguineus*, also indicated that the relationships of that family were with the Tuberaceae.<sup>18</sup> In a schematic arrangement, however, Millardet actually placed the Myriangiaceae between the family Onygenaceae and the Pyrenomycetes. The Onygenaceae are a family of the Plectasceineae of Fischer (11), who appended to this order the family Myriangiaceae, then including only the genus *Myriangium*. In Montagne and Berkeley's (6) original report of *M. duriaei* it was suggested that the organism resembled *Dothidea* as well as certain lichens. In 1909 Von Höhnelt (16, p. 351) placed the family Myriangiaceae in the Dothideales, near which order the family had been placed by Starbäck (35) in 1899, when he raised it to ordinal rank. Theissen and Sydow (42, p. 6) placed the order Myriangiales in the Dothidiaceae, containing the two other orders Dothideales and Pseudosphaeriales. Arnaud (1, p. 653, 677) stated that the order appeared to consist of primitive pyrenomycetes possibly derived from the Plectascales. He placed the order before the Microthyriales, with which he considers the Dothideales have developed in a parallel line.

#### PATHOGENICITY

In his brief description of the genus *Elsinoe*, Raciborski stated that its members consisted of parasitic ascomycetes producing galls on the host plants. Such parasitism for *Elsinoe canavaliae* is not doubted by the present writer. Arnaud, after examining the material of *E. canavaliae* collected in Java by Raciborski,<sup>19</sup> indicated that the organism was here growing on another fungus within the galls rather than being the direct cause of these malformations. In one statement, however, he suggested that the galls were actually caused by *E. canavaliae*, or, as he termed the fungus, "*Uleomyces*

<sup>18</sup> Tuberineae Fischer (10).

<sup>19</sup> Op. cit. (See footnote 3.)

*canavaliae*." His assumption of the parasitism of the organism on another fungus and not on *Canavalia* may have been influenced in part at least by the fact that Hennings (12, p. 107), in describing the type species of the genus *Uleomyces*, i. e., *U. sanguineus*, or, as termed by him, "*U. parasiticus*," had stated that it was parasitic on the ascomycete *Parmularia styracis*, which occurs on *Styrax*. Hennings based his description on a specimen gathered in Brazil in 1892 by Ule, in whose honor the genus is named. As already indicated, he later recognized that the same organism, discovered in Paraguay by Balansa in 1883, had been described by Spegazzini under the name *Phymatosphaeria sanguinea*. In describing the fungus under this name Spegazzini reported it on living *Styrax*. On the label of the specimen just cited, as well as of another specimen of *Uleomyces sanguineus* examined by Arnaud<sup>20</sup> it is indicated that the fungus is growing on leaves of *Styrax*. This material was collected at Sao Leopoldo, Brazil, in 1904. *Parmularia styracis* is also present on these leaves, and as already indicated, this organism rather than the *Styrax* leaf is considered by Arnaud to be the host of the *Uleomyces*. Representative illustrations of the specimen just cited, as well as of another specimen or species of *Uleomyces*, *U. decipiens* Syd.<sup>21</sup> on *Pasania glabra*, Japan, 1901, are shown in Plates 3, B and C, and 4, B and C, respectively. They illustrate the practically certain foliar origin of the conspicuous ascoma in *Uleomyces*. The general appearance of *U. decipiens* is shown in Plate 4, A and B, while in Plate 3, A, D, E, and F, as labeled, is shown the general appearance of fructifications of both *U. sanguineus* and *Parmularia styracis*, as well as a section through a fruit body of the latter fungus showing its attachment to the leaf.

#### SUMMARY

This paper contains most of the available data concerning a little-known disease of *Canavalia*, here termed "scab," which is said to affect *C. gladiata* and *C. ensiformis*. It is pointed out that on account of the confusion between these two species, as well as between *C. ensiformis* and *C. luzonica*, it is not definitely known at present whether *C. ensiformis* is actually susceptible to its attack. On the basis of scabbed material of *Canavalia* from Singapore in the United States National Herbarium, the Malay Peninsula is added to the known range of the disease. Previously this included Ceylon, the Philippines, and Java, in which country, so far as known, the disease was discovered, as well as first reported by Raciborski about 30 years ago.

Other supplementary data presented deal with the symptoms of the disease, the characteristics of its causal fungus, *Elsinoe canavaliae*, and the status of the classification of this organism.

Microconidia or other conidia representing the hitherto unreported conidial stages of *Uleomyces sanguineus* and *Myriangina mirabile* are reported, these being similar to those here noted for the first time in *Elsinoe canavaliae*, but previously seen in *Spheceloma symphoricarpi* and *S. fawcettii*. The occurrence of the 2-layered ascus in *Myriangina* is reported.

<sup>20</sup> Sao Leopoldo, Brazil, 1904, J. Rick. (RICK, J. FUNGI AUSTRO-AMERICANI, Fasc 3, No. 49) (32, p. 309), labeled *Ascomycetella sanguinea* (Speg.) Sacc.

<sup>21</sup> Japan, 1921, T. Yoshinga. (SYDOW. FUNGI EXOTICI EYSICATI, Fasc. 2, No. 513) (37).

The data are presented for reference in connection with investigations of myriangioid fungi, but primarily because of their direct bearing on scab of *Canavalia*, which seems to be of some economic importance, as well as on a destructive disease of Lima bean now being investigated.

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# LIMA-BEAN SCAB CAUSED BY ELSINOE<sup>1</sup>

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## INTRODUCTION

This paper embodies the results of the writer's recent study of a destructive fungous disease which affects both bush and pole Lima bean (*Phaseolus lunatus macrocarpus* Benth.) in Cuba and Porto Rico, but which is not known to occur in the United States. The material on which the work was based consisted of diseased leaves, stems, and fruits of Lima beans, collected in November, 1929, and during the late winter and early spring of the past three years at Wajay and Hoyo Colorado, Cuba, by Prof. S. C. Bruner and Sr. Cecilio Aguiar, and also of green unshelled Lima beans intercepted at the ports of New York and New Orleans and in Porto Rico by inspectors of the United States Plant Quarantine and Control Administration. A few additional specimens representing Cuban shipments were taken from markets at Washington, D. C., by G. G. Becker, of the Plant Quarantine and Control Administration, and by the writer. One specimen taken by the Federal Horticultural Board in 1923 had been deposited in the Mycological Collections of the Bureau of Plant Industry. This was intercepted from a Cuban shipment entered at the port of New York. All the other specimens were collected between January 17 and March 30, 1930. Fully 100 specimens were intercepted at New York from Cuban shipments entered at that port; the 1 from New Orleans, also of Cuban origin, was taken from ship's stores. Of the 4 specimens from Porto Rico, 3 were from shipments about to be sent to New York, while the fourth was from the planting from which one of these shipments had come.

A similar if not the same disease affects Canavalia in Ceylon, Java, and the Philippines (9),<sup>3</sup> but even though possibly identical with it, the Lima-bean disease is here discussed separately. Most of the available data pertaining to the Canavalia disease have been published by the writer (9) with the explanation that this was done because of their direct-bearing on the Lima-bean disease.

<sup>1</sup> Received for publication July 21, 1930; issued January, 1931. A summary of this account, amplified from a paper read before the Botanical Society of America, Mycological Section, December 30, 1929, appeared in the following publication: JENKINS, A. E. A SCAB DISEASE OF LIMA BEAN IN CUBA AND PORTO RICO. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rpt. 14: 6-97. 1930. [Mimeographed.] It was previously referred to (8) under the title "Lima-Bean Scab Caused by *Elsinoe canavaliae* Rac."

<sup>2</sup> Thanks are due Prof. S. C. Bruner, Departamento de Fitopatologia y Entomologia, Estacion Experimental Agronomica, Santiago de las Vegas, and Sr. Cecilio Aguiar, Chief Inspector, Seccion de Sanidad Vegetal, Secretaria de Agricultura, Comercio y Trabajo, Cuba, for specimens on which were based most of the studies here reported; and to R. R. Sasser and H. S. Dean, of the Plant Quarantine and Control Administration, U. S. Department of Agriculture, for many additional specimens and for the information which is summarized in Table 1. The color readings, which are based on Ridgway,<sup>4</sup> as well as the color drawings, are by J. Marion Shull. Photographs and photomicrographs are by J. F. Brewer. The sections were made by means of the freezing microtome.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 22

<sup>4</sup> RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C., 1912.



## THE DISEASE

## NAME

Cuban growers of the Lima bean refer to the disease under study as "verruca."<sup>5</sup> Anthracnose is the name commonly applied to diseases of similar etiology or symptoms (8, 10, p. 46-47). Stoneman (19) and Shear (16) have already noted that this word was designed for the familiar disease of grape which it connotes. Shear called attention to the fact that it was originated by Fabre and Dunal (7) in 1853 in connection with their discussion of that disease. It could thus appropriately signify the legume disease or diseases mentioned, except that, as first applied by Scribner (15, p. 361) in 1888, it is already in common use to denote the legume disease caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Br. and Cav. It was with these facts in mind, as well as because of its resemblance to citrus scab, that the writer (9) proposed for the Canavalia disease the name scab rather than anthracnose. With this fuller explanation, the term "scab" is here employed in referring to the Lima-bean disease.

## HISTORY AND IMPORTANCE

The earliest records at hand of the observation of Lima-bean scab are those furnished by specimens of scabbed Lima bean collected in Cuba between 1914 and 1916 by J. R. Johnston and the specimen intercepted by the Federal Horticultural Board in 1923. In submitting the first specimen for diagnosis, Professor Bruner stated that the disease had been brought to his attention several years previously by Sr. Cecilio Aguiar and that Cuban plant inspectors had confused it with bean anthracnose. As to the importance of the disease, he stated that the spotting greatly disfigures the pods, which may themselves be destroyed when the tender peduncles are attacked, and that where grown for export the spotted pods are separated as culls, thereby causing considerable loss to the grower. He<sup>6</sup> wrote that the plantings at Hoyo Colorado, Cuba, from which specimens were forwarded in March, 1930, were more severely attacked than any he had yet observed. He stated:

The Lima beans, pole variety, had been planted in a banana field and thus received more shade than usual. A careful count was not made, but a conservative estimate is that not less than 75 per cent of the pods were diseased. \* \* \* It was discovered that the disease not only disfigures the pods by the unsightly spots, but that it causes many of them to wither, die, and fall off, or prevents them from filling out and developing normally. This is due to the infection of the tender stems of the young pods, producing a slow strangling effect. \* \* \* Many half-grown pods which had been killed in this way, as well as others in a more or less withered condition \* \* \* were observed still clinging to the vines. \* \* \* The spots occurred quite commonly on the outer exposed side of the pod, and at times on the highest point on the plant where there are no overhanging leaves or branches.

On July 10, 1930, Professor Bruner wrote that there had been a general increase of affected plants during the current year, that practically all the commercial fields in some provinces were affected, and that the disease was the most important of those attacking the crop in Cuba.

Although severely scabbed, the green Lima-bean fruits received from the Plant Quarantine and Control Administration during the ship-

<sup>5</sup> Data contained in letter from Professor Bruner dated Mar. 10, 1930.

<sup>6</sup> Letter dated Mar. 7, 1930.

ping season just past (November, 1929, to March, 1930) were generally thrifty and well filled, and in no case were the seeds affected. Only occasional lesions of the anthracnose were seen, and comparatively few of other diseases. Upon learning that the first two samples, received on January 17 and 21 (N. Y. Nos. 13799 and 13815), were affected by scab, the New York representative of the administration reported that the disease had been more or less prevalent in all shipments from Cuba during the present importing season, and furthermore, that intercepted green Limas from Cuba, showing what appeared to be the same disease, had "in the past years \* \* \* been diagnosed as affected by anthracnose." The specimen referred to earlier as intercepted in 1923, which was labeled *Colletotrichum lindemuthianum*, as well as other records of the Federal Horticultural Board and of the Plant Quarantine and Control Administration, had already revealed this to be the case. Some of these records have been published (20, 21).

February and March, 1930, are the only months for which the Plant Quarantine and Control Administration has figures that indicate the prevalence of scab in green unshelled Lima beans entered at the port of New York from Cuba. These figures, which are incomplete for March, are presented in Table 1, which also contains the amount and distribution of green unshelled Limas from the same source during the other three months of the shipping season just past. The totals of scabbed shipments for March do not include several consignments in which scab was reported as present by the inspectors of the administration, but for which specimens were not submitted to the Washington office. Additional information shows that the number of scabbed pods in individual shipments received from Cuba or Porto Rico during the past shipping season varied from a small quantity to 100 per cent. For example, the number of infected pods in nine Cuban shipments, or 4,936 containers, received in March, ranged from 5 to 90 per cent, and in the shipments from Porto Rico, from 10 to 15 per cent. During the entire shipping season a total of 113 intercepted shipments of beans on which the disease was present were received from ports distributed as follows: 108 from Cuba through New York, 1 from Cuba through New Orleans, and 4 from Porto Rico through San Juan.

TABLE 1.—*Lima beans entered at the port of New York from Cuba from November, 1929, to March, 1930, inclusive, and prevalence of scab on February and March shipments*

Month	Shipments	Containers in shipments	Containers examined		Samples of diseased beans taken	Samples affected by scab	Shipments represented by scabbed samples	Containers in shipments from scabbed samples
			Number	Per cent				
November.....	69	10, 016	-----	-----	-----	-----	-----	-----
December.....	131	29, 491	-----	-----	-----	-----	-----	-----
January.....	104	10, 536	-----	-----	-----	-----	-----	-----
February.....	139	23, 587	474	2	87	82	79	18, 445
March.....	98	24, 431	454	2	21	20	19	8, 131

## SYMPTOMS

The presence of the disease may usually be recognized by the characteristically thickened, raised, or swollen lesions first appearing on the young, growing parts of the plant. Including their histologic structure, not described in this paper, they are of the same general character and appearance as those of scab of *Canavalia* (9). The following description is based upon laboratory studies of fresh material received from Cuba and Porto Rico, supplemented by certain field observations by S. C. Bruner.

## LEAF SPOT

The leaf lesions are generally more pronounced on the upper leaf surface than on the lower, apparently differing in this respect from scab of *Canavalia*. They are often not noticeably thickened. From a few to several hundred (pl. 1, A and B) may occur on one leaf. These may be scattered over the surface of the blade or grouped along the veins. They are usually circular, ranging from minute dots to areas about 4 mm. in diameter. Those near the veins, however, are sometimes considerably larger and elongated in the direction of the veins. They may be convex on the upper surface and concave on the lower. (Pl. 2, A and D.) In color they are often a uniform vinaceous buff.<sup>7</sup> Certain appreciably thickened lesions, however, were liver brown in part and discolored only on the upper surface. Sometimes the leaf tissue about the spots is killed. (Pl. 1, B.) Diseased leaves may be distorted or they may become broken and torn as a result of the breaking away of affected parts. (Pl. 1, B.) Lesions on the petioles are rough and pinkish brown and may involve the entire surface or only a part.

## STEM CANKER

On the stems (pl. 1, C and D) the individual lesions or cankers are often somewhat raised, ranging from minute spots to elliptical or elongate areas 1 cm. or more in length, with the longer diameter parallel to the axis of the shoot. None of the individual lesions examined completely surrounded the stem, although where confluent they not uncommonly encircle it for a distance of at least 2 cm. In color the lesions on this part of the plant are generally vinaceous buff, like many of the leaf lesions. They are sometimes bordered by a purplish-brown band of varying width.

## POD SPOT

The pod lesions constitute the most noticeable symptoms of the disease. Individual lesions on green fruits are irregular, elliptical, or subcircular, ranging from mere flecks to areas 5 to 8 mm. or sometimes somewhat over 1 cm. in greatest diameter. The line of the

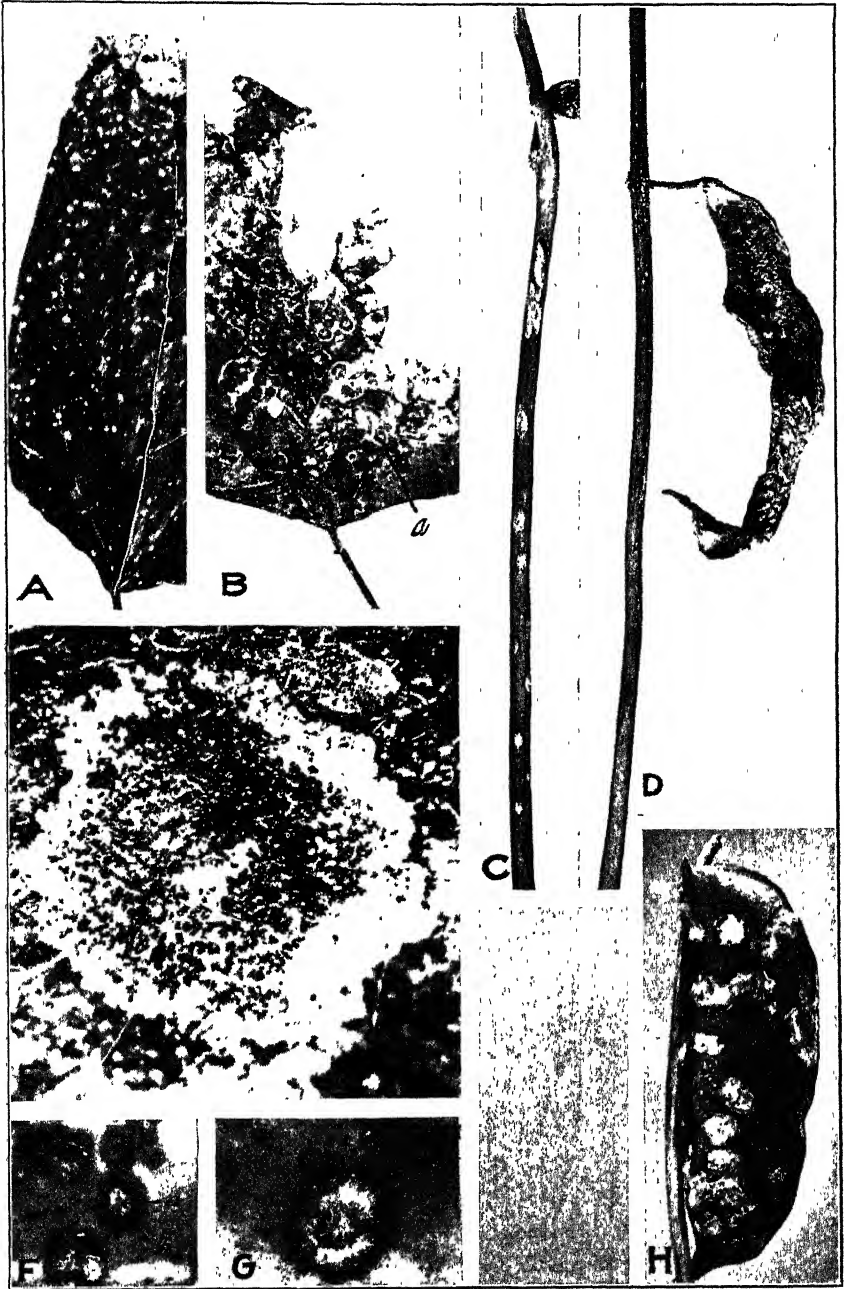
<sup>7</sup> RIDGWAY, R., op. cit.

## EXPLANATORY LEGEND FOR PLATE 1

A-E.—Scab lesions on leaves (A, B, E), stems (C, D), and young fruit (D), from S. C. Bruner, Hoyo Colorado, Cuba, March, 1930. A-D,  $\times 1$ ; E (enlargement of B, *a*),  $\times 23$ .

F, G.—Scab lesions on parts of two full-grown green fruits, from Cuba through United States Plant Quarantine and Control Administration. Light-colored central region with broad purplish border is shown in F, *a* and b.  $\times 2$ .

H.—Scab lesions on a full-grown green fruit, from S. C. Bruner, Wajay, Cuba, late winter or early spring of 1928.  $\times 1$ .



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

longer axis commonly extends obliquely across the pod from the lower suture toward the apex. The lesions are often distributed irregularly over the pod surface, although not uncommonly they occur in greatest abundance on but one valve; they may be grouped toward the apex of the pod or along one or both sutures. (Pl. 3, A, and pl. 1, H.) Lesions on one valve near either suture may involve the other valve to a limited extent, but more often they do not extend across the suture. When not circular, the longer diameter of such lesions not infrequently parallels the suture. (Pl. 1, H.) Where lesions are numerous or confluent, the entire surface of the fruit, or sometimes only one valve, may be affected.

Lesions may not be noticeably raised, and occasionally they are somewhat sunken. Often, however, they assume the form of a definite swelling, sometimes depressed at the center and there checked in a stellate manner, exposing the green tissue of the pod. (Pl. 1, G.) Groups of lesions arranged along the edges of the sutures may so deform the margins of the pods that they resemble the notched edge of a saw. Even where sufficiently depressed to cause a bulging of the surface immediately beneath, none of the lesions examined had penetrated the ovary wall; in fact they have been scarcely or not at all visible on its inner face.

The most conspicuous symptom of the disease is the swollen, brightly colored lesions on the pods. (Pl. 3, A.) The color varies noticeably, not only in spots of different ages but also in those of practically the same age. For example, immature lesions often range from brick red to Kaiser brown, while others, somewhat older, are light cinnamon drab bordered with diamond brown, or dark Indian red bordered with Etruscan red. Somewhat older (pl. 1, F) or fully mature lesions (pl. 1, H) may be avellaneous to lighter at the center, with a border varying from Indian red to maroon. Confluent lesions are often variegated. Other colorations of the lesions occasionally seen are Morocco red and Napal red.

## THE FUNGUS

### IDENTITY, HISTORY, AND CLASSIFICATION

The fungus causing scab of Lima bean is here tentatively identified as *Elsinoe canavaliae* Rac. (13), the cause of scab of Canavalia. This ascomycete was discovered on the Asiatic sword or saber bean (*C. gladiata* (Jacq.) DC.) at Buitenzorg, Java, by Raciborski (13), who reported as well as described it in 1900, making it the type of the genus *Elsinoe*, now classified in the Myriangiales. The present writer (9) has explained that although reported in Ceylon and the Philippines on jack or horse bean (*C. ensiformis* (L.) DC.), a species "native to the West Indies and the adjacent mainland" (12), and very closely allied to the sword bean, it is not clear from the literature whether this is actually attacked by *Elsinoe canavaliae*. Additional data pertaining to the history and classification of the pathogene are

### EXPLANATORY LEGEND FOR PLATE 2

A-D.—Sections through leaf lesions showing ascomata on upper side of lesion. A (arrow), group of conidiophores; C, a, ascus probably of subepidermal origin. From S. C. Bruner, Hoyo Colorado, Cuba, March, 1930. A-C,  $\times 380$ ; D,  $\times 130$ .  
E.—Conidia developed on the surface of ascospores. From Cuba through United States Plant Quarantine and Control Administration between January 17 and March, 1930.  $\times 380$ .

contained in the writer's (9) publication dealing with scab of Canavalia. It is noteworthy that no scab was found on the jack bean growing between the rows of Lima bean from which were harvested the scabbed Lima-bean fruits shipped from San Juan, P. R. This statement is based upon the writer's examination of leaves and stems of jack bean from that field, forwarded by the inspector of the Plant Quarantine and Control Administration who discovered there the scabbed condition of the Lima beans.

Much further investigation will be required, however, before it can be definitely determined whether or not the two closely similar legume diseases here discussed are caused by the same species of *Elsinoc*. Very few data are available pertaining to the morphology of *E. canavaliae* on Canavalia. No fully mature or living material from this host has been available to the writer, nor have there been observed other than microconidia representing its *Sphaeceloma* conidial stage (9). In so far as corresponding morphological characteristics may be compared, however, the *Elsinoc* on Lima bean is certainly indistinguishable from that on Canavalia. Even its orientation in the host tissues is essentially the same. Based upon the specimens that have been available for examination, there appears to be this difference in host reaction, however; in the case of Canavalia the more commonly infected leaf surface appears to be the lower, and in that of *Phaseolus lunatus macrocarpus* the upper.

The Lima bean is regarded as native to South America (4, 12). It seems pertinent to note Piper's (12) report on the occurrence of "wild forms \* \* \* in Cuba, Porto Rico, and Guadeloupe, from Mexico to Panama, and in Colombia, Venezuela, Brazil, and Peru, and doubtfully in Argentine." E. D. Merrill<sup>8</sup> states that this legume is now naturalized and is common and widely distributed in the Philippines; that it was introduced from tropical America at an early date; and that it occurs in those regions in the Philippines where sword bean, jack bean, and other species of Canavalia are grown.

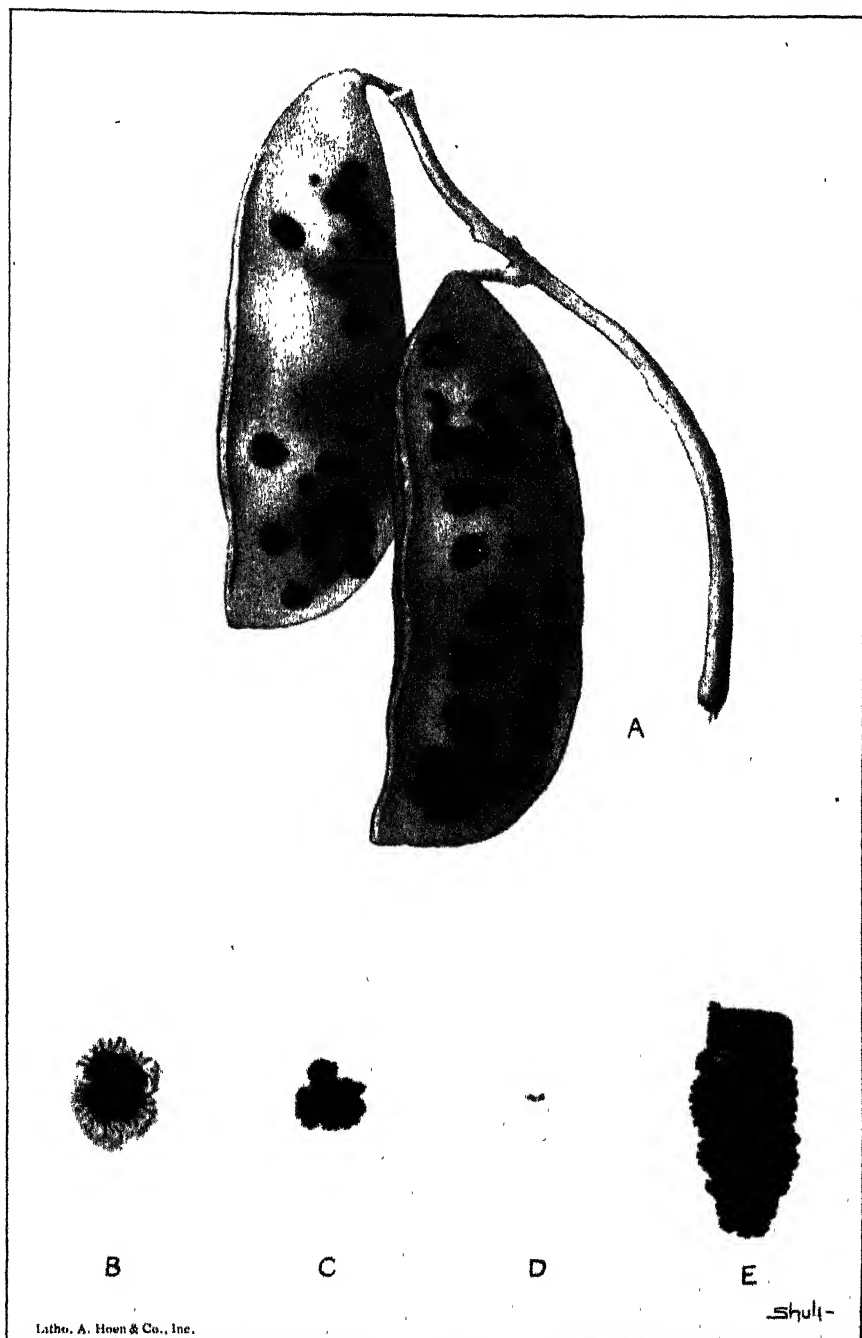
In the lists that record *Elsinoc canavaliae* on sword and jack bean in the Philippines (2, 3, 14, 22) and in Ceylon (5, 6) no mention is made of this fungus on the Lima bean, although a number of other fungi are reported on that host. The taxonomic and physiologic significance of these data remains to be determined. Assuming that the *Elsinoc* on Lima bean and on sword bean are identical, as is done in this account, there is the possibility that they represent physiologic forms; that is, the fungus from one host may not infect the other.

#### MORPHOLOGY

##### HYPHAE

Hyaline hyphae or stromata of the *Elsinoc* are shown in Plate 4, A, b, B, a, and C, c. These preparations represent sections through the outer part of young lesions on fresh pods. The first section is from a pod placed for a few days in a moist atmosphere; the other two were made on the day the material was received. The orientation of the hyphae here corresponds exactly to that described by Arnaud (1) for their distribution in leaf lesions on Canavalia; that is, they have grown between the phellem (pl. 4, A, c) and the epidermis, as well as within the disrupted epidermis and the outermost

<sup>8</sup> Letter dated Mar. 10, 1930.



A.—Fresh Lima-bean fruits showing scab lesions. From Cuba, received through United States Plant Quarantine and Control Administration between January 17 and March, 1930.  $\times 1$ .

B-E.—Four-weeks-old (B-D) and ten-weeks-old (E) cultures of *Elsinoe canavialis* on potato-dextrose (B), beef (C), corn-meal agar (D), and potato-plug (E) media. Isolated from infected pods sent by A. Reimer from Wajav, Cuba, November, 1929.  $\times 1$ .





broken cells of the phellem. In some instances, however, hyphae penetrate the phellem and phellogen for some distance. The walls of the subepidermal hyphae shown in Plate 4, B, *a*, have become almost completely gelatinized. Where more or less exposed the hyphae or stromata become dark. (Pl. 5, D, and K, *b*, and pl. 2, B-D.) Suggestive of *Phyllachora*, they are then often visible as conspicuous brownish or black coverings or patches on the surface of the lesions. (Pl. 1, E.) On account of the formation of the perfect stage of the fungus in the region immediately beneath, they may be arched, pebbled, or broken. (Pl. 5, D and K.) As explained in the following paragraph, however, the dark coloration of the fungus on the surface of the lesions may also be due to the development there of the conidial stage.

#### CONIDIAL STAGE

##### CONIDIOPHORES

The conidiophores of the *Elsinoe* soon become colored, as is characteristic of the form genus *Sphaceloma*. They generally develop from the hyaline intraepidermal hyphae in the central region of recently formed lesions (pl. 4, B, *b*, and C, *b*) or in the younger or marginal zone (pl. 5, H) of older lesions. Sometimes they form a more or less continuous dark layer over the surface of the stroma. (Pl. 2, A, arrow.) In size they range from a few to  $20\mu$  in length by about  $4\mu$  in width. They correspond to the vertical hyphae observed by Millardet (11, p. 12, pl. 3, fig. 27) and Starbäck (17, p. 42, pl. 1, fig. 33) as arising from the surface of the ascoma in *Myriangium duriae* Mont. and Berk., and in *M. thallicolum* Starb., respectively; they were not interpreted by these authors as representing conidial fructifications.

##### CONIDIA

Conidia are probably readily produced from the conidiophores under favorable conditions. In the material examined, however, only a few were seen actually attached to the conidiophores. These consisted of hyaline spherical microconidia identical with those recently reported for this species on *Canavalia* (9) and ovoid or elliptical pale-colored conidia about  $10\mu$  by  $4\mu$  in size. Younger or hyaline conidia of the latter type, that is, the kind usually reported for the genus *Sphaceloma*, have as yet not been seen on the Lima-bean host. They were abundant, however, in young agar cultures (pl. 4, D-F), which will be referred to later in the section dealing with the cultural studies of the fungus. The elliptical 1-septate structure shown in Plate 4, A, *a*, is a swollen conidium. Although they probably occur, Coniotheciumlike conidia, such as those reported by the writer (9) for *Sphaceloma symphoricarpi* Barrus and Horsfall, have not as yet been seen in *Elsinoe canavaliae*.

#### PERFECT STAGE

##### ASCI

In the description of the genus *Elsinoe*, of which *Elsinoe canavaliae* is the type, it is explained that perithecia or receptacles (disci) are lacking and that the asci are generated at intervals in the subepidermal stroma. The asci are said to develop singly or in groups, sometimes with their walls in contact. On Lima bean not only are

they distributed as just described (pl. 2, B-D; pl. 4, C, *a*; pl. 5, A, D, and I), but they may also form within the epidermis. The group of asci shown in Plate 2, C, *a*, is probably subepidermal in origin. Where asci are exposed, as frequently occurs, they may form a hymenium simulating the Exoascaceae; occasionally they are present in the same pustules with the conidiophores. Where they break through the epidermis (pl. 4, C, *a*), they may simulate *Magnusiella*, near which Raciborski (13) classified his new genus *Elsinoe*. In some instances there were seen hyphae apparently leading up to the bases of the asci as illustrated by Arnaud (1, pl. 10, J) for *Atichia millardeti* Rac. In an individual group the asci may be in practically the same stage (pl. 5, A, K, *c*, and I) or in different stages of development (pls. 2, B; 4, C, *a*; and 5, D). The preparation of the fungus shown in Plate 5, A, represents an ascoma, similar to that illustrated in Plate 5, D, as dissected from the surface of a lesion, transferred to a slide, and then crushed under a cover glass.

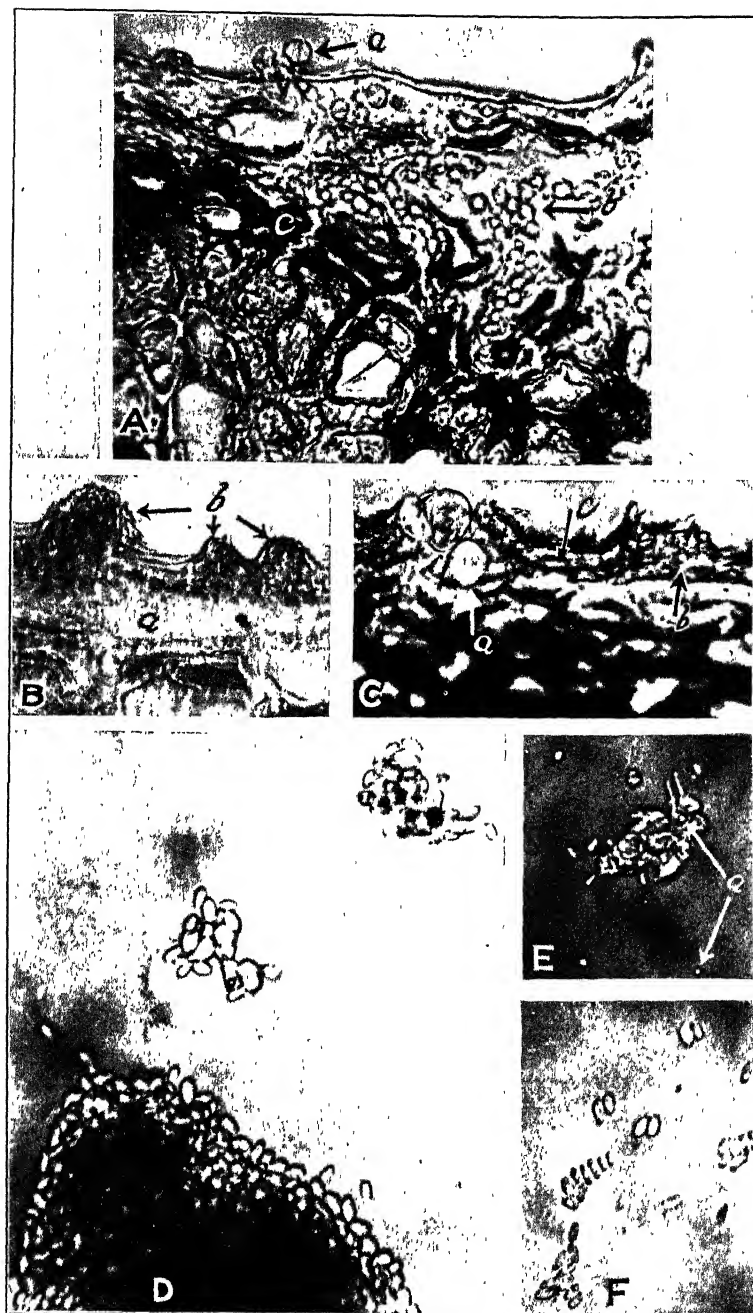
The largest asci seen in *Elsinoe* on Lima bean have been somewhat larger, as well as more stipitate, than those observed in this fungus on Canavalia. Those of which the wall was still intact reached  $40\mu$  in length by  $30\mu$  in width. Here the outer inelastic primary membrane was observed to have become ruptured (pl. 5, A, *a*, E, and I) allowing the thickened inner or secondary layer to expand in the form of a clavate ostiolate structure often twice the length of the original ascus; sometimes it was entirely separated from the primary membrane. (Pl. 5, F.) The region of greatest elongation appears to be the thickened apical part as seen in the undifferentiated ascus. (Pl. 4, C, *a*, and pl. 5, D.) The double wall of the ascus in this genus of the Myriangiales has not been reported previously, nor has it been made the subject of special study by the writer. Its interpretation here as such and the terminology employed are those of Millardet (11) in regard to homologous structures in *Myriangium duriaei*. Stevens and Weedon (18) and others have presented diverse interpretations of these structures in the Myriangiales.

In studying the *Elsinoe* on Lima bean it was observed that, instead of containing ascospores, the apparently undifferentiated ascus was sometimes filled with microconidia. These are seemingly freed in a body, since in some preparations there were seen free spherical conidia in groups which still retained the shape of the ascus cavity, although larger than that space because of the swelling of the conidia.

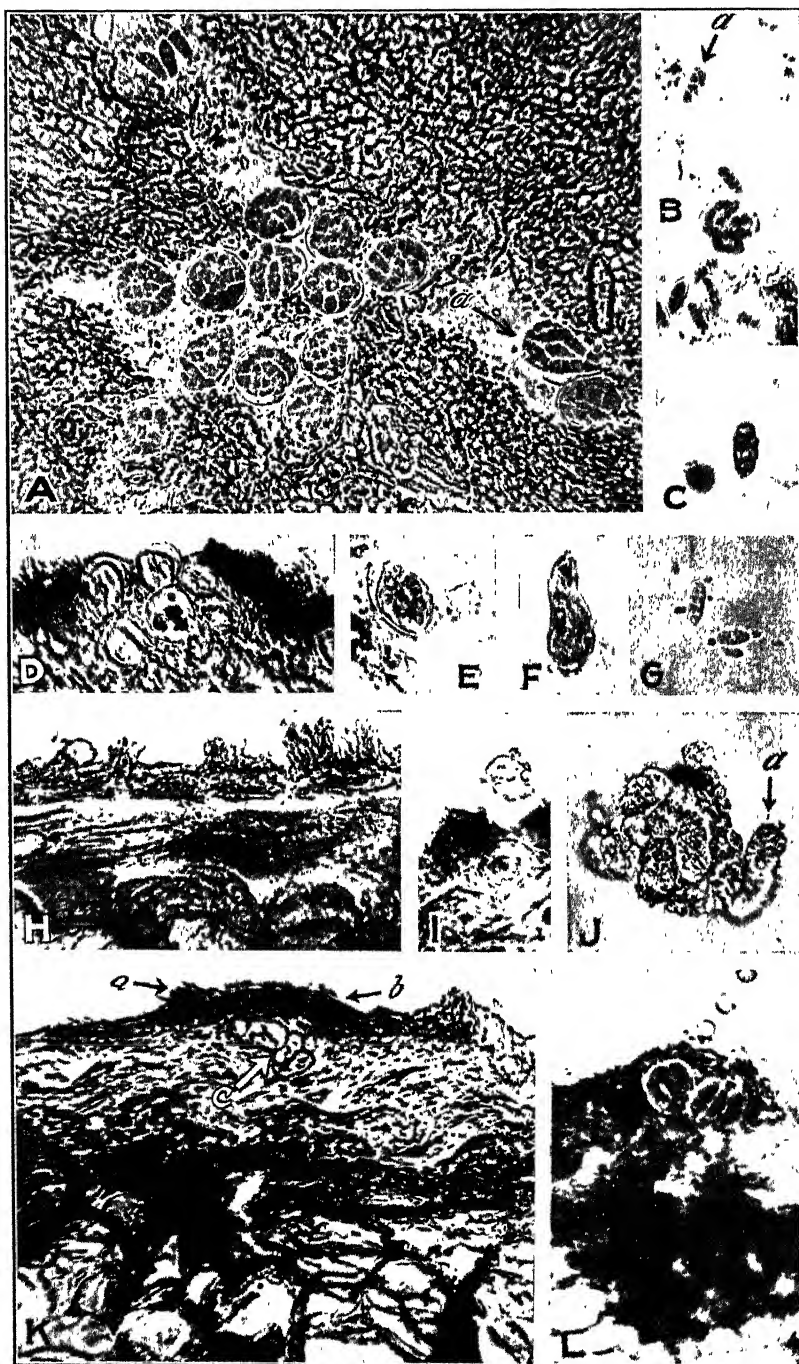
In one instance, in which an infected pod was placed in a moist atmosphere, small yellowish masses at length appeared on the surface of the lesions. No spores or conidia were present in them. They may have been composed of the matrix in which the spores were produced in the ascus, together with the secondary layer possibly extruded in connection with the discharge of spores from the asci in situ in the lesion. The forcible ejection of spores from asci was proved by so placing parts of fresh valve lesions in the cover of an inverted

#### EXPLANATORY LEGEND FOR PLATE 4

- A-C.—Sections through outer part of young pod lesions showing hyphae or a stromatic mass (A, *b*; B, *a*; and C, *c*); acervuli (B, *b* and C, *b*); swollen septate conidium (A, *a*); phellem layer (A, *c*); and perithecia breaking through the epidermis (C, *a*). Material from S. C. Bruner, Wajay, Cuba, November, 1929.  $\times 380$ .
- D-F.—Production of conidia in 2 day-old agar cultures inoculated with particles of a stromatic mass from an old agar culture; colonies in D photographed in situ without cover glass; E, *a*, refringent granules.  $\times 380$ .



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Petri dish containing agar that the spores could be shot upward on the agar surface. The cover was lined with moist filter paper, and the inoculum elevated by placing under it one or two glass slides. In Plate 2, E, are shown eight ascospores or conidia produced from them as thus discharged on the surface of an agar plate during an 18-hour period and apparently from a single ascus.

#### ASCOSPORES

The brown as well as muriform ascospores (pl. 5, B, *a*, and C) not previously observed, although inferred by Arnaud (1) to occur in *Elsinoe canavaliae*, have been seen in this fungus on Lima bean. Their occasional red coloration may be due to staining from the coloring matter in the diseased tissues in which was borne the ascoma containing them. The ascospores, which like the conidia may become greatly swollen (pl. 5, J), commonly produce spherical (pl. 5, J, *a*) microconidia or larger ovoid or elliptical conidia. Those shown in Plates 5, G, and 2, E, represent, respectively, development in a water culture within 30 minutes and in a glycerin-agar culture within an 18-hour period. The conidia in the agar culture practically mask the asci from view. The greatly swollen spores shown in Plate 5, J, are from a pod lesion held for 24 hours in a moist chamber. These ascospores, like the eight that were ejected on the agar plate, are probably from a single ascus. The production of spherical microconidia was also observed to take place from young ascospores in situ in the ascus, as reported by the writer (9) for *Myriangina mirabile* (P. Henn.) Von Höhnelt.

#### CULTURAL CHARACTERISTICS

In Plate 3, B-E, are illustrated 4-week-old slant cultures of the fungus grown on potato-dextrose, beef, and corn-meal agar media and on 10-week-old potato-plug medium. Conidia were not present in any quantity, if at all, in these test-tube cultures. Ovoid or elliptical, mostly biguttulate, conidia were abundant, however, in a 2-day-old culture made by inoculating the surface of a freshly poured corn-meal agar plate with small particles of stromatic growth from such cultures. (Pl. 4, D-F.) Conidial formation was apparently here stimulated by the transfer of the fungus to a more moist atmosphere than that in which it had formerly been growing. Although conidia were not present in old agar cultures, they were numerous on the surface of colonies or sporodochia developed from 5-day-old tissue cultures. They were then mostly swollen; some were 1-septate and developing germ tubes. These cultures were made by placing por-

#### EXPLANATORY LEGEND FOR PLATE 5

- A.—Crushed preparation of an ascoma on surface of pod lesion. *a*, Perithecium showing outer membrane ruptured.  
 B-C.—Ascus and hyaline ascospores (B), a muriform ascospore (B, *a*), and a reddish-brown ascospore (C).  
 D.—Section through ascoma on surface of pod lesion.  
 E.—Ascus showing ruptured primary membrane.  
 F.—Expanded secondary layer of ascus.  
 G.—Production of conidia from ascospores in water culture.  
 H.—Acervuli in marginal region of pod lesion.  
 I.—Ascus on surface of lesion, inner part of which has separated from the thin transparent primary layer, at its base an immature ascus and at left dark stroma.  
 J.—Greatly swollen ascospores producing microconidia (*a*).  
 K.—Section through pod lesion showing acervulus (*a*) rising from the intraepidermal hyphae (*b*), beneath which is a group of young asci (*c*).  
 L.—Well-developed spores in situ within asci in ascoma.  
 Material from S. C. Bruner, Wajay, Cuba, late winter and spring of 1928 and 1929. All  $\times 380$ .

tions of disinfected pod lesions on the surface of agar in a Petri dish. Isolations were made from ascospores as well as from tissue cultures. Refrangent granules present in some of the hyphal cells composing the stromata in the test-tube cultures (pl. 4, E, a) correspond to those observed by Millardet (11) in *Mycrangium duriaei*.

### SUMMARY

A fungous disease, called scab, affects Lima bean in Cuba and Porto Rico. This disease was formerly confused with bean anthracnose. A specimen of scabbed Lima bean collected in Cuba between 1914 and 1916 constitutes the earliest available record of the disease. Although the pathogene has probably been transported many times into the United States, it is not known to be established here. Earlier record of its occurrence in Porto Rico has not been found.

In the field, leaves, stems, and fruits are affected, and when severely diseased the fruits may fail to develop. In no case in the Lima-bean fruits examined, including those of marketable size, have the lesions penetrated to the seed. This disease is of economic importance not only because of its ravages in the field but also on account of the loss in marketing due to the conspicuous disfiguration of the pods. It is the most important disease of the crop in Cuba.

The symptoms of the disease, together with the morphology and cultural characteristics of the fungus, are discussed.

The pathogene is tentatively identified as *Elsinoe canavaliae* Rac. This species has been known, heretofore, only in Java, Ceylon, the Malay Peninsula, and the Philippines, on species of *Canavalia*.

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# FACTORS INFLUENCING THE EFFECTIVENESS OF ORGANIC MERCURY DUSTS IN PEA-SEED TREATMENT<sup>1</sup>

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## INTRODUCTION

The results of treating pea seed with various chemical substances for the control of disease and for seed protection through the germination period have been reported by the writer.<sup>2</sup> The conclusions, based on experimental data obtained in the field and in the greenhouse, were as follows:

In general, the treatment of pea seed with organic mercury dusts has increased the percentage stand of plants. The amount of this increase was variable, being greater on seed of low vitality and when seed was planted under soil conditions of high-moisture content and low temperature. It appears that the treatment of pea seed with organic mercury dusts will usually increase the stand of plants sufficiently to justify their use under soil conditions that prevail at planting time in New York.

From the experiments mentioned above it was evident that there were at least two distinct phases of seed treatment to be considered: (1) The value of seed treatment in the control of seed-borne diseases, and (2) the protection of the seed from decay caused by soil-borne organisms prior to the germination of the seed and the establishment of the young seedling.

The value of seed treatment for the control of some of the seed-borne diseases of pea has been reported by the writer<sup>3</sup> and summarized as follows:

In these experiments the use of organic mercury dusts on seed infected with *Ascochyta pisi* did not control primary infection from diseased seed to the above-ground parts of the plant. Preliminary experiments tend to show that treatment of seed infected with *Mycosphaerella pinodes* or *Ascochyta pinodella* is of considerable benefit in reducing foot-rot injury.

Haenseler<sup>4</sup> did not notice any appreciable decrease in the amount of *Ascochyta* foot rot in Semesan-treated pea plots.

In the experiments conducted by the writer in 1926 and 1927 it was noted that various environmental factors, such as temperature and moisture content of the soil at the time of planting and immediately after planting, and the nature of the treating material greatly influenced the results obtained. Similar effects have been noted by Haenseler,<sup>5</sup> who showed that where conditions for germination were less favorable Semesan-treated peas gave an increase of 1.5 to 11 per

<sup>1</sup> Received for publication Aug. 5, 1930; issued January, 1931. This paper reports work done at the New York State Agricultural Experiment Station and at the Washington Agricultural Experiment Station. It is published with the approval of the directors of both stations as paper No. 169, College of Agriculture and Experiment Station, State College of Washington.

<sup>2</sup> JONES, L. K. STUDIES OF THE NATURE AND CONTROL OF BLIGHT, LEAF AND POD SPOT, AND FOOT ROT OF PEAS CAUSED BY SPECIES OF ASCOCHYTA. N. Y. State Agr. Expt. Sta. Bul. 547, 46 p., illus., 1927.

<sup>3</sup> JONES, L. K. Op. cit.

<sup>4</sup> HAENSELER, C. M. EFFECT OF ORGANIC MERCURY SEED TREATMENTS ON GERMINATION AND YIELD OF PEAS. N. J. Agr. Expt. Sta. Ann. Rpt. 48: 232-238. 1925.

<sup>5</sup> HAENSELER, C. M. Op. cit.

cent in stand as compared with untreated seed. Clayton<sup>6</sup> also obtained a better stand with several kinds of supposedly healthy vegetable seed by treating them with organic mercury compounds, particularly when the seed was planted under unfavorable conditions.

The present paper records the results of experiments made for the purpose of determining (1) the relative value of various organic mercury dusts in the treatment of healthy seed peas and (2) the effect of soil temperature and soil moisture on the effectiveness of the seed-treatment compounds.

#### RELATIVE EFFECTIVENESS OF SEED-TREATMENT MATERIALS

In 1928 field experiments were conducted in the vicinity of Geneva, N. Y., to compare the relative effectiveness of various organic mercury dusts and other seed-treatment materials. Special organic mercury dusts containing different percentages of chlorphenol mercury, nitrophenol mercury, and cresol mercury on carriers of talc, clay, or lime were compared to ascertain with which concentration and inert ingredient the best results would be obtained.<sup>7</sup> These dusts were compared with other seed-treatment materials. A list of the materials used is shown in Table 1.

Three varieties of peas—Green Admiral, Alaska, and Advancer were used in the experiments, and three plantings of each variety were made. The plantings were made on different dates, beginning April 27 and extending to May 10. Fifty 1,000-seed lots of each variety in triplicate were measured and treated in February. The seed was treated by shaking it in a paper bag with the dust materials, which were used at the rate of 3 ounces of dust to a bushel of seed. At the time of planting, each 1,000-seed lot was placed in the hopper of a mechanical seed planter and three 30-foot rows were planted. The seeds that remained in the hopper, varying in number from 15 to 95, were counted. Thus, 905 to 985 seeds were used in each test. The planter was so adjusted that the seeds were dropped approximately 1 inch apart in the rows. Quite uniform spacing of the seed was obtained. The depth of planting was about 1½ inches.

Counts on the emergence of plants were made when the seedlings were 2 to 3 inches high. The results are recorded in Table 1. Records of rainfall and of soil moisture and temperature 2 inches below the surface were kept during the planting season and are correlated with planting dates in Figure 1.

Except in the case of Green Admiral, series A, which was planted on April 27, the soil moisture and temperature were favorable for germination of the seed. This is shown by the average emergence of the untreated seed, which varied from 49 to 70 per cent. Under these favorable conditions for germination the minimal benefit was derived from seed treatment. The Green Admiral, series A, showed a very marked reduction in stand of plants, which may be correlated with the heavy rainfall of April 28 and 29.

<sup>6</sup> CLAYTON, E. E. INCREASING STANDS FROM VEGETABLE SEEDS BY SEED TREATMENT. N. Y. State Agr. Exptl. Sta. Bul. 554, 16 p., illus., 1928.

<sup>7</sup> The special organic mercury dusts were furnished by C. R. Orton of the Bayer Co. (Inc.), New York City.

TABLE 1.—Results of field germination tests of treated and untreated healthy seed peas, Geneva, N. Y., 1928

Test No.	Treatment and material used *	Percentage organic mercury as—		Solubility of mercury	Inert material	Germination of peas of different varieties planted on dates shown											
		Cresol				Green Admiral		Alaska		Advance							
		Chlorophenol	Nitrophenol			Series A, May 9, Apr. 27		Series B, May 9		Series C, May 7		Series A, May 10		Series B, May 10		Series C, May 10	
						Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1	Control					27	55	63	66	58	46	61	58	32			
2	do					32	53	63	66	65	42	61	63	53			
3	do					22	52	58	68	60	42	61	38	38			
4	Bayer dust			Low	Talc.	50	56	65	70	48	51	69	59	54			
5	Bayer 143		1	do	Clay	31	48	64	66	60	49	58	54	59			
6	Bayer 124		4	High	Talc.	30	57	58	67	59	51	67	57	57			
7	Bayer 126		4	do	Clay	27	54	60	71	61	64	68	58	54			
8	Bayer 133		4	Low	Talc.	29	55	64	65	66	60	60	58	58			
9	Bayer 135		4	do	Clay	33	52	70	69	68	47	64	61	61			
10	Bayer 120		4	High	Talc.	40	57	66	66	55	53	66	60	60			
11	Bayer 107		4	do	Clay	34	49	70	68	70	56	66	59	60			
12	Bayer II-6		6	Low	Talc.	35	65	73	67	67	56	70	60	60			
13	Control					25	52	68	64	60	53	61	58	61			
14	Bayer 132		6	Low	Clay, talc	23	48	66	67	60	48	66	61	61			
15	Bayer 125		6	High	Talc.	24	48	63	65	62	54	58	61	61			
16	Bayer 127		6	do	Clay	17	64	65	64	63	51	66	54	54			
17	Bayer 123		6	Low	Clay, talc	18	49	72	74	70	53	62	58	58			
18	Bayer 128		6	High	Clay	22	49	74	73	68	71	72	64	64			
19	Bayer 205			do	Talc.	21	47	70	68	66	64	47	63	63			
20	Bayer 206			do	do	19	46	70	64	66	62	55	55	55			
21	Bayer 207			do	do	24	51	71	67	59	51	65	58	58			
22	Bayer 111		2	do	do	26	52	75	67	64	62	50	71	71			
23	Control		2	Low	do	29	48	66	61	59	50	68	56	56			
24	Bayer 112		3	Low	Talc.	35	56	76	56	67	67	58	63	63			
25	Semesan Jr.		12.0	Medium	Lime	42	58	83	55	57	64	70	64	64			
26	Semesan		35	High	do	46	67	84	59	65	55	73	69	69			
27	Bayer 142		3	Medium	Clay	35	59	76	64	62	65	64	64	64			
28	Bayer 110		5	High	do	35	58	80	70	66	65	64	64	64			
29	Uspulun liquid, 0.25 per cent for 1 hour.					53	63	67	66	64	46	61	57	57			
30	Uspulun		30	High		41	68	80	73	70	60	68	68	68			
31	Mercuric chloride, 1/1000 for 15 minutes					32	50	62	66	64	49	55	46	46			

<sup>a</sup> Series A and series B were planted on the Nester farm. Series C was planted on the experiment station farm. Weather records in fig. 1 are from the Nester farm. The results obtained in each series are presented in the sequence of planting in the field. Laboratory germination: Green Admiral, 95 per cent; Alaska, 98 per cent; Advance, 97 per cent.

<sup>b</sup> All dust materials were used at the rate of 3 ounces to 1 bushel of seed. They were thoroughly mixed with the seed by shaking in a paper bag.

TABLE 1.—Results of field germination tests of treated and untreated healthy seed peas, Geneva, N. Y., 1928—Continued

Test No.	Percentage organic mercury as—			Germination of peas of different varieties planted on dates shown									
	Treatment and material used		Solubility of mercury	Inert material		Green Adminal		Alaska		Advanceer			
	Chlorophenol	Nitrophenol				Series A, Apr. 27	Series B, May 9	Series C, May 8	Series A, May 4	Series B, May 9	Series C, May 7	Series A, May 4	Series B, May 10
						Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
22	Bayer 150	2	6	Medium	Clay	31	63	81	72	68	58	52	70
23	Control					28	55	74	62	66	48	45	59
24	Bayer 154	6	2	Medium	Clay	39	56	73	61	67	71	45	57
25	Bayer 161	10		High	Clay	30	55	78	68	68	64	54	70
26	Seuensen Bel	10		High	Clay	42	56	72	73	67	62	51	75
27	Bayer 210			Low	Clay	33	63	67	70	62	66	56	68
28	Bayer 210	8		Medium	Talc	45	69	67	64	69	71	52	69
29	Calomel, 100 per cent				Clay	35	65	75	67	71	64	55	70
30	Calomel, 50 per cent				Clay	32	62	79	62	66	53	51	67
40	Calomel, 10 per cent				Talc	24	57	66	75	71	70	47	70
41	Bayer 221	5.5	High			33	62	81	65	65	55	45	75
42	Gray Wax					31	61	69	66	69	50	32	62
43	Copper-oxehloride					33	52	71	66	68	56	32	67
44	Control					31	57	76	65	63	74	45	40
45	Cupro-fabonite					31	64	75	56	63	72	49	48
46	Copper carbonate					24	50	62	61	63	67	42	59
47	Du Pont K. I. F. c					33	76	65	57	67	72	67	66
48	Du Pont K. I. C. c					32	75		57	60	63	52	67
49	Control					31	57		53	64	60		59
50	do.					42			57				34
51	do.												
Average of controls						29	73	70	61	66	61	47	62

c 1.5 per cent of ethyl mercury chloride plus absorbent plus clay filler.

d 1.5 per cent ethyl mercury chloride plus clay filler.

The most consistently beneficial results from seed treatment were obtained with Uspulun dust or Semesan dust. It appears that organic mercury dusts containing less than 6 per cent chlorphenol mercury or nitrophenol mercury were relatively ineffective in protecting the seed from decay-producing organisms, and that dusts containing 12 per cent or more were most effective.

The tests to determine the relative value of lime, clay, and talc as carriers and the relative value of dusts of high and low solubility showed variable results. Differences obtained in these tests are in general within the range of the mathematical probable error. Dusts having higher percentages of organic mercury should have been used, since the tests show that dusts containing 6 per cent or less of

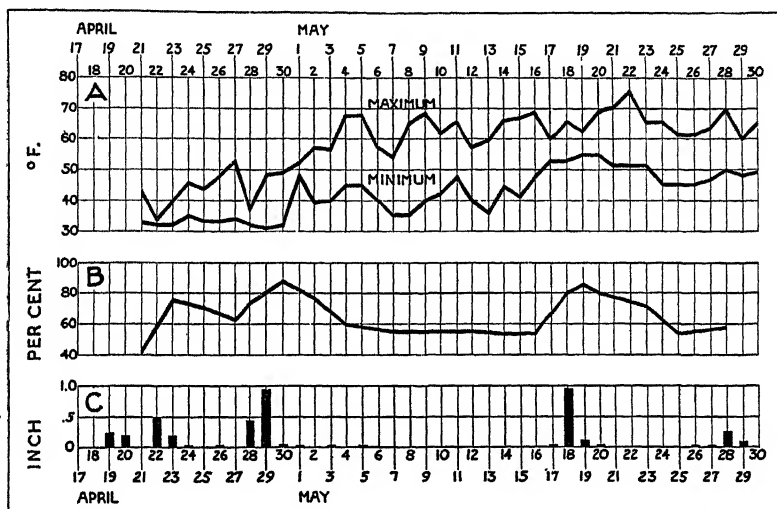


FIGURE 1.—Summary of various factors used in interpreting results of seed-treatment experiments, Geneva, N. Y., 1928: A, daily maximum and minimum soil temperatures 2 inches below the surface; B, moisture in soil as average percentage of moisture-holding capacity 2 inches below the surface; C, precipitation

organic mercury were relatively ineffective. As in previous experiments,<sup>8</sup> less beneficial results were obtained from treatment of seed of the Alaska variety than seed of the sweet wrinkled varieties. In the nine trials the average increase in percentage stand of plants due to treatment with Semesan dust and Uspulun dust was 10 and 11 per cent, respectively.

#### INFLUENCE OF SOIL MOISTURE ON EFFECTIVENESS OF SEED-TREATMENT MATERIALS

From the data appearing in Table 1 and from observations on previous similar experiments it was evident that excessive soil moisture immediately after planting is an important factor in determining the value of seed treatment. With this fact in mind, several series of experiments were conducted in the greenhouse at the agricultural experiment station, State College of Washington, Pullman, Wash., in 1928 and 1929.

<sup>8</sup> JONES, L. K. Op. cit.

All of the greenhouse experiments were conducted in Palouse silt loam soil brought into the greenhouse from fields where peas had never been grown. The soil had a moisture-holding capacity of 36 to 40 per cent, and prior to planting efforts were made to have the moisture content of the soil at 55 to 65 per cent of its moisture-holding capacity. In the following experiments the seeds were planted about 1 inch apart in the rows and 2 inches deep in the soil. Treated and untreated rows of each lot of seed were alternated in the benches. The seeds to be treated were shaken in a paper bag with Semesan dust, which was used at the rate of 3 ounces to a bushel of seed. In the experiments where water was added after planting, about three-fourths of an inch of water was sprinkled on the soil immediately after the seed was planted, and the soil was not again watered until the seedlings began to emerge. The temperatures at which the experiments were conducted were quite constant, fluctuating from 2 to 4 degrees, as the four sections of the greenhouse were equipped with thermostatic temperature-control apparatus.

TABLE 2.—*Effect of watering immediately after planting upon germination of Semesan-treated and untreated pea seed, Pullman, Wash., 1928<sup>a</sup>*

Variety and treatment	Stand of plants <sup>b</sup>		Variety and treatment	Stand of plants <sup>b</sup>	
	Soil not watered	Soil watered		Soil not watered	Soil watered
Green Admiral. <sup>c</sup>	Per cent	Per cent	Horsford Market Garden. <sup>c</sup>	Per cent	Per cent
Control. . . . .	49	34	Control	67	46
Semesan treated	83	77	Semesan treated	85	77

<sup>a</sup> Seed was planted in 60° F. greenhouse Nov. 28, 1928.

<sup>b</sup> Each figure is the average of four 100-seed rows.

<sup>c</sup> Laboratory germination: Green Admiral, 95 per cent; Horsford Market Garden, 86 per cent.

Table 2 gives the results of the first plantings of Green Admiral and Horsford Market Garden seed obtained from seedsmen at Rigby, Idaho. In the soil watered immediately after planting the emergence of plants from untreated seed was 15 to 21 per cent less than that of plants in the unwatered soil. The treatment of the seed with Semesan was of marked benefit in each case but of greater benefit where the soil was watered after planting.

Further tests in which the same experimental procedure was used were made with 50 lots of seed produced in the vicinity of Fairfield, Wash., in 1928. The results of these tests (Table 3) are similar to those obtained in preceding experiments, and further emphasize the reduction in emergence that may occur if the soil is watered soon after planting. These trials also show the hardier character of the Alaska seed as compared with that of the sweet wrinkled varieties. With the 36 lots of Alaska seed having an average laboratory germination of 96.4 per cent there was an average decrease of 34 per cent in emergence due to watering the soil immediately after planting. In the unwatered soil an increased emergence of 14 per cent was noted from the Semesan-treated seeds. In the soil that was watered soon after planting an increased emergence of 41 per cent followed Semesan treatment of seed. The results with the sweet wrinkled

varieties were somewhat similar. Greater benefit followed treatment of the seed of these varieties in unwatered soil than was the case with the Alaska variety.

TABLE 3.—*Effect of watering immediately after planting upon germination of Semesan-treated and untreated pea seed, Pullman, Wash., 1929*<sup>a</sup>

Variety or type and treatment	Average laboratory germination	Stand of plants	
		Soil not watered	Soil watered
Alaska <sup>b</sup>	Per cent	Per cent	Per cent
Control.....	96.4	75±1.50	41±1.88
Semesan treated.....		89±0.65	82±1.04
Winkled <sup>c</sup>	88.0	44±1.88	22±2.96
Control.....		70±2.94	65±3.09
Semesan treated.....			

<sup>a</sup> Tests conducted in 60° F. greenhouse in January and February.

<sup>b</sup> Average of thirty-six 100-seed lots.

<sup>c</sup> Probable error of the mean,  $PE_m = \frac{SD \times .6745}{\sqrt{n}}$ .

<sup>d</sup> Average of fourteen 100-seed lots.

When it was found that watering soon after planting reduced the stand of plants, an experiment was planned to ascertain the amount of water necessary to influence germination and the effect of applying it soon after planting as compared with the effect of delaying the application for 24 or 48 hours. Three benches, 4 by 13½ feet, were each divided into five equal parts after the soil had been thoroughly mixed. A fairly uniform moisture content of 60 per cent of the moisture-holding capacity was obtained in the beds. Division of each bench was accomplished by means of wooden partitions extending from the floor of the bench to well above the surface of the soil. Fifty treated seeds and 50 untreated seeds from each of four lots were planted in each division of the three benches. The row of 50 untreated seeds of each lot was planted beside the row of 50 treated seeds from the same lot. The 15 divisions or plots were identical as to soil, soil moisture, source of seed, and plan of planting. A uniform temperature of 67° to 70° F. was maintained during the experiment. In each bench the first division received no water until the plants emerged; the second received one-fourth inch; the third, one-half inch; the fourth, three-fourths inch; and the fifth, 1 inch of water. The water was applied as uniformly as possible with a sprinkling can. In one bench the application was made immediately after planting, in another 24 hours afterwards, and in the third 48 hours afterwards. The data obtained are reported in Table 4.

With untreated seed of the Alaska variety very marked decreases in germination occurred in those plots to which one-half to 1 inch of water was applied immediately after planting, but one-fourth inch of water had no detrimental effect. It appears that the application of one-fourth to 1 inch of water 24 hours after planting had relatively little detrimental effect on germination of untreated Alaska seed and that the application of one-fourth to three-fourths inch of water 48 hours after planting increased the stand of plants from untreated seed. In these experiments the amount of water applied and the time of application appeared to have little effect on the germination of Semesan-treated Alaska seed. A slight reduction in stand from the



Semesan-treated seed was noted where 1 inch of water was added immediately after planting. In the 15 tests the average stand of plants from treated Alaska seed was 93 per cent, while that from untreated Alaska seed was 62 per cent.

TABLE 1—*Relation of the time of water application to the effectiveness of Semesan treatment of pea seed, Pullman, Wash., November, 1929<sup>a</sup>*

Variety and treatment	Stand of plants					
	Watered immediately after planting		Watered 24 hours after planting		Watered 48 hours after planting	
	Control	Semesan treated	Control	Semesan treated	Control	Semesan treated
Alaska <sup>b</sup>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
No water	66±3.31	60±0.00	66±2.21	96±0.00	66±1.93	92±1.23
1/4 inch water	66±3.89	88±1.23	60±7.27	94±1.98	86±2.28	98±0.00
1/2 inch water	18±4.18	62±0.64	60±2.51	96±0.00	86±0.84	92±0.00
3/4 inch water	32±1.92	66±0.91	56±4.69	94±0.91	76±1.23	96±1.11
1 inch water	31±2.21	80±1.79	51±1.11	94±0.97	66±1.58	92±0.84
Surprise <sup>d</sup>						
No water	35	68	20	68	40	84
1 in. water	20	42	28	54	48	82
1/2 in. water	20	58	12	60	26	76
3/4 in. water	0	16	8	52	28	68
1 in. water	0	32	10	66	22	72

<sup>a</sup> Experiments conducted in greenhouse at 67°-70° F. Moisture-holding capacity of soil, 23 per cent, soil contained 60 per cent of its moisture-holding capacity; 50 seeds per row test, seed planted 2 inches deep.

<sup>b</sup> Each figure is the average of three 1-row tests, 1 row each of 3 different lots of seed.

<sup>c</sup> Probable error of the mean,  $PE_m = \frac{SD \times .6745}{\sqrt{n}}$ .

<sup>d</sup> Each figure is the percentage stand from one 1-row test.

Much better stands were obtained from the Alaska than from the Surprise seed. This difference was especially noticeable with the untreated seed and with the application of one-half to 1 inch of water even 48 hours after planting. Marked increase in stand of plants followed Semesan treatment in all the tests. The Semesan treatment was less effective, however, in protecting the Surprise seed from decay than in protecting the Alaska seed. This was especially noticeable where three-fourths to 1 inch of water was applied immediately after planting.

#### INFLUENCE OF SOIL TEMPERATURE ON EFFECTIVENESS OF SEED-TREATMENT MATERIALS

Further experiments were conducted to determine the effect of soil temperature on germination and on value of seed treatment. The results are reported in Table 5. These experiments were conducted at the same time as those reported in Table 2, and with seed from the same lots. The best germination of untreated seed was obtained at 60° F., with a reduction in stand of Green Admiral of 19 and 24 per cent at 45° and at 70°, respectively, and of 19 and 20 per cent in the stand of Horsford. Very marked increases in germination followed the Semesan treatment of the seed, with similar increases regardless of soil temperature. At 60° and 70° the plants emerged 6 to 8 days after planting, while at 45° they required 20 to 28 days for emergence.

TABLE 5.—*Relation of soil temperature to germination of Semesan-treated and untreated pea seed, Pullman, Wash., 1928*<sup>a</sup>

Variety and treatment	Stand of plants in—		
	45° green-house	60° green-house	70° green-house
Green Admiral <sup>b</sup>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Control	30±2 77	49±0 05	25±4 28
Semesan treated	64±1 46	83±0 84	82±1 92
Horsford Market Garden, #	48±1 44	67±2 31	47±3 49
Semesan treated	65±0 71	85±0 84	68±3 68

<sup>a</sup> Seed planted in greenhouse Nov. 28, 1928.<sup>b</sup> Each figure is the average of four 100-seed rows.<sup>c</sup> Probable error of the mean,  $PE_m = \frac{SD \times 6745}{\sqrt{n}}$ .<sup>d</sup> Laboratory germination: Green Admiral, 95 per cent; Horsford Market Garden, 86 per cent.

## SUMMARY

In a series of eight hundred 1,000-seed tests with various seed-treatment materials, organic mercury dusts containing at least 12 per cent of the mercury phenolate were the most effective in increasing the stand of plants. Uspulun dust and Semesan dust gave the most consistently beneficial results. Organic mercury dusts containing 6 per cent or less of chlorphenol mercury, nitrophenol mercury, or cresol mercury were of little value in increasing the stand of plants.

The experimental data presented show that the application of water to the soil soon after planting greatly reduces the percentage germination of untreated pea seed and that this reduction is greater with the sweet wrinkled varieties than with the Alaska variety. Marked reduction in stand of plants resulted from the application of one-half to 1 inch of water immediately after planting, while the application of water 24 or 48 hours after planting had little effect upon the germination of the untreated Alaska seed.

An increase in germination resulted from treating the seed with Semesan dust under various soil-moisture and soil-temperature conditions. In general, the sweet wrinkled varieties were more benefited by the treatment than the Alaska variety. With the Alaska variety, seed treatment appeared to assure a good stand of plants regardless of the moisture and temperature conditions of the soil.

The data presented in this paper indicate that the variations in soil moisture near the time of planting and differences in soil temperature cause wide variations in the results obtained from seed treatment. Such variations may account to a large extent for the contradictory statements found in the literature on the value of seed treatment.



# EFFECT OF DRYING AND SULPHURING ON VITAMIN C CONTENT OF PRUNES AND APRICOTS<sup>1</sup>

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## INTRODUCTION

The results of a study of the vitamin C content of dried fruits reported from these laboratories some months ago (8)<sup>2</sup> gave details only in regard to peaches, but, as noted in that report, similar tests with prunes and apricots, involving a larger variety of drying conditions, were also made.

So far as the writers have been able to determine, no previous study, except that of Eckman (2), has been reported on the antiscorbutic property of apricots fresh or dried, and only two on prunes, those of Hess and Unger (4) and Eckman (2). Hess and Unger found practically complete loss of vitamin C in dried prunes, as did Eckman in dried apricots and prunes, but in none of the tests was a comparison made with the fresh fruit, nor were any details of the drying process given. In the study of the antiscorbutic property of peaches referred to above (8), all of the fruit was from the same orchard, and the fresh fruit was tested along with the sundried and dehydrated products, sulphured and unsulphured. The sulphured peaches, both sundried and dehydrated, were found to have retained the full antiscorbutic value of the fresh fruit, but the unsulphured products apparently retained none. The principal questions raised by this finding were: (1) Does sulphur dioxide protect other fruits equally well? (2) If so, how much is required for such protection? and (3) How is the protective effect achieved? The present report undertakes to answer in part, at least, each of these questions.

## PREPARATION OF THE FRUIT

The fruit was prepared as previously described (8, 9), under the direction of members of the staff of the fruit products laboratory. Prunes of the Agen (French) variety from the 1927 and 1928 crops, grown in California, were preserved by pitting and grinding, packing in 8-ounce tin cans, and freezing solid. The frozen fruit was stored at  $-17^{\circ}\text{C}$ . until fed to the test animals. It is assumed that the vitamin C content of fruit preserved in this way is not changed even after long storage. Delf (1) found that frozen orange juice retained its antiscorbutic efficiency after one year in storage, and preliminary tests in this laboratory with frozen orange juice of several types confirm this finding.

<sup>1</sup> Received for publication Aug. 25, 1931, issued, January 1930. Contribution from the laboratory of household science and the fruit products laboratory, College of Agriculture, University of California.

<sup>2</sup> The writers are indebted to the Dried Fruit Association of California for much of the fruit used in this study and for moisture and sulphur dioxide determinations made by R. S. Hiltner and B. F. Hatherell.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 45.

TABLE 1 -- Preparation and composition of fruits tested

Fruit and crop	Lot No.	Method of preparation	Moisture	Net shrinkage	pH	Sulphur dioxide content
			Per cent <sup>a</sup>	Per cent		Parts per million
Agen. prunes, 1927 crop	10a	Pitted, ground, frozen, and kept at -17° C.	79.5	1.0		
	11a	Dehydrated whole at 72° C. for 30 hours after lye dipping, washing, and sulphuring overnight	11.7	1.0	3.1	88
	12a	Same as for 11a, but without sulphuring	12.9	3.9		
Agen. prunes, 1928 crop	18a	Lye dipped, washed, sundried 7 days, held in stack 7 days	14.3	3.9	3.3	
	10	Same as for 10a	62.6	1.0	3.5	
	11	Same as for 11a	19.0	2.1	3.2	1,980
	12	Same as for 12a	18.2	2.2	3.5	
	13	Same as for 11a, but without lye dipping	18.6	2.2	3.2	1,020
	14	Sulphured overnight, whole, sundried 7 days, held in stack 7 days	19.8	2.1	3.1	1,005
	15	Same as for 11a, but without lye dipping or sulphuring	21.4	2.1	3.3	
	16	Same as for 14, but without lye dipping or sulphuring	20.2	2.1	3.6	
	17	Same as for 18a, but sulphured overnight	17.8	2.2	2.6	2,600
	18	Same as for 18a	17.0	2.2	3.2	
	19	Same as for 18a, but held at room temperature	17.4	2.2		
Royal apricots, 1928 crop.	5	Pitted, ground, frozen, and kept at 17° C.	82.9	1.0	3.5	
Royal apricots, 1929 crop.	5a	Pitted, ground, evacuated, released with nitrogen, sealed cold, frozen, and kept at -17° C.	85.7	1.0	3.7	
	1a	Dehydrated for 17 hours at 65° C.	12.8	6.1	3.6	
	2a	Same as for 1a, but previously sulphured 1 hour.	15.6	5.9	3.7	892
Royal apricots, 1928 crop.	3a	Same as for 1a, but previously sulphured 6 hours.	13.8	6.0	3.5	2,028
	1	Cut, pitted, sulphured 3½ hours, dehydrated 24 hours at 72° C.	18.8	1.7	2.4	515
	2	Same as 1, but sulphured 30 minutes	17.0	4.8	2.9	125
	3	Same as 1, but unsulphured	22.6	1.5	3.2	100
	4	Same as 1, steamed 3½ minutes, and sulphured 20 minutes.	16.3	1.9	3.2	80
	6	Cut, pitted, sulphured 2½ hours, sundried 11 days.	19.0	4.7	3.3	700
	7	Same as 6, but sulphured 30 minutes	17.5	4.8	3.1	170

<sup>a</sup> Flesh.

The dehydrated and sundried prunes were prepared as indicated in Table 1, and as previously described. The effect of dipping the fruit in lye was of particular interest in this inquiry, and several preparations were made with and without this treatment. All dried products were stored at 0° C.

Royal apricots of the 1928 and 1929 crops were similarly prepared, and, as shown in Table 1, an attempt was made to obtain products of different sulphur dioxide content. The frozen fresh apricots of the 1928 crop were found to have lost practically all antiscorbutic value (Table 5), and at the same time on thawing they were observed to evolve a good deal of gas, presumably air and carbon dioxide retained in the fresh tissue. Accordingly, the experiment was repeated in 1929 with similar fresh apricots, but precautions were taken to exclude air. The fruit was pitted, ground, and packed in a large jar which was evacuated and the vacuum released with nitrogen. The pulp was then packed quickly in 8-ounce tin cans, the lids given the first crimp, evacuated, and the vacuum again released with nitrogen, after which the usual freezing and storage procedure was carried out. Excellent preservation of vitamin C resulted in this product. (Table 5.) It was interesting to find this exception to the effectiveness of the ordinary freezing process. If the sharp

freezing of fruits should assume importance as a means of preservation, attention should be directed to this danger and suitable evacuation provided for fruits, such as apricots, in which retained gases are likely to promote the destruction of vitamin C.

The experience of Kohman and Eddy (7) with the respiratory oxygen of canned apples appears to have been similar to that of the writers with frozen apricots. However, preliminary experiments in this laboratory with canned apricots do not indicate the persistence of oxygen retention during processing.

A similar destruction of vitamin A was not observed in unevacuated frozen apricots used in a previous study (9). An evacuated lot (LS) from the same crop yielded no higher values for vitamin A than did the unevacuated. Apparently vitamin A is less susceptible to oxidation than is vitamin C, or the apricots do not contain as potent specific catalysts for destruction of vitamin A as for vitamin C.

The moisture, hydrogen-ion concentration, and sulphur dioxide content of the fruit samples were determined by the methods previously described (9, *footnote 1*). It should be noted that samples 2, 3, and 4 of the apricots shown in Table 1 were essentially un-sulphured, so far as retained sulphur dioxide is concerned. Un-sulphured fruit usually has an apparent sulphur dioxide content of 50 to 100 parts per million. Differences in sulphur dioxide content found by a single method within this range are therefore probably not significant.

#### METHODS OF TESTING FOR VITAMIN C

A modification of the diet proposed by Sherman, LaMer, and Campbell (12), as previously described (8), was used in all these tests. The basal diet contained rolled oats, 69 per cent; baked skim-milk powder, 30 per cent; and sodium chloride, 1 per cent. In addition, 2 c. c. of cod-liver oil was fed daily to each animal separately. The enlargement of rib joints in apparently nonscorbutic guinea pigs mentioned by Schwartze, Murphy, and Hann (11) may well have been caused by the lack of antirachitic vitamin in the basal diet which they used. It was to avoid this danger that cod-liver oil was substituted for butterfat, which was originally included. The animals were kept on the basal diet with tomato juice for several days, or until they showed a definite and rapid increase in weight, before the fruit doses were fed.

The record of the large number of negative controls accumulated over a period of seven years of continual vitamin C testing leaves little doubt as to the scurvy-producing character of this diet. Moreover, long-continued feeding of positive controls on this diet, with a daily supplement of 12 c. c. of canned tomato juice, together with the reproduction records and the rearing of second generation animals, makes the writers equally confident that the diet is complete except for the antiscorbutic factor.

TABLE 2 - Comparison of condition of guinea pigs after 60 and 90 days feeding on fresh and dried apricots and prunes

Fruit and lot No.	Amount fed daily	Animals	Average weight			Average gain or loss, per day		Final condition
			Initial	After 60 days	After 90 days	In 60 days	In 90 days	
	Grams	Number	Grams	Grams	Grams	Grams	Grams	
Dehydrated sulphured apricot, No. 1	3	2	317	315	216	0	-1.1	Mild scurvy
	4	5	329	196	513	2.8	2.4	No scurvy
	6	2	361	587	649	3.7	3.2	Do
Sundried sulphured apricot, No. 6	6	1	340	438	526	1.6	2.0	1, mild scurvy, 3, normal
	8	2	356	408	355	.9	0	1, mild scurvy, 1, normal
	10	1	321	376	471	.9	1.7	Mild scurvy
Fresh frozen prune, No. 10	12	1	328	377	391	.8	.7	3, mild scurvy, 1, normal
	15	5	351	356	387	0	.4	3, mild scurvy, 2, normal
	20	2	359	472	454	1.9	1	No scurvy
Dehydrated sulphured prune, No. 11	3	1	338	191	512	2.6	2.3	Do
	8	3	326	481	555	2.6	2.5	Do
Sundried sulphured prune, No. 17	5	1	333	391	431	.9	1.1	3, no scurvy, 1, mild scurvy
	8	3	323	492	422	2.8	1.1	2, no scurvy, 1, mild scurvy

In the earlier series of experiments, including all tests with fruit of the 1927 and 1928 crops, the 90-day test period was used. At the end of the 90 days autopsy was performed on all animals and careful examination was made for the usual pathological lesions of scurvy. It was observed, however, that there was little change in the condition of even border-line cases after the sixtieth day, and, as shown in Table 2, there was little change even in the rate of growth during the first 60 days as compared with that of the entire 90-day period. Eighty seven per cent of all the animals which were only partly protected from scurvy by the smaller fruit doses, and which died before the 90-day period was over, died previous to the sixtieth day. (Table 3.) Most of the remaining 13 per cent had shown definite symptoms of scurvy by the sixtieth day. There seemed little likelihood, therefore, that accuracy would be sacrificed by reducing the period of test feeding to 60 days. Accordingly, this was done in testing the 1929 apricot preparations.

TABLE 3. Survival period of scorbutic guinea pigs when fed insufficient doses of fresh frozen and dried prunes, and fresh frozen and dried apricots, as antiscorbutics

Fruit	Animals showing scurvy, due to insufficient dosage	Animals dying previous to sixtieth day		Animals dying previous to sixtieth day and after sixtieth day		Average survival period
		Number	Per cent	Number	Per cent	
Prunes, 1927 crop (all products)	16	13	81	3	19	46
Prunes, 1928 crop (all products)	12	37	88	5	12	38
Apricots, 1928 crop, fresh frozen (not evacuated)	28	24	86	4	14	16
Dried apricots, 1928 and 1929 crops	41	36	88	5	12	39
Total	127	110	87	17	13	41
Negative controls	12	12	100			29





Zilva (3, 14, 15) in a long series of vitamin C studies used chiefly the 60-day period, and Sherman, LaMer, and Campbell (12) in their original communication report 70 to 90 days as the feeding period. Since the daily growth of the animals on the test doses was practically the same during the 60-day period (Table 3) as during the 90-day period, and since even incipient scurvy manifests itself before the sixtieth day, there can be little reason for carrying the test longer than 60 days even for the best quantitative comparisons possible with the biological method.

Another precaution has, however, been introduced in the histological examination of the teeth of all test animals, following Höjer's suggestion (5). Some guinea pigs on each of the 1929 apricot doses were killed after 14 days and their teeth examined for destruction of dentine and changes in odontoblasts. Animals on the same doses were killed after 60 days and similar examinations of their teeth were made. The results of these studies are not, however, reported here.

The "scurvy score," often mentioned by investigators of the vitamin C content of foods, has been included in all autopsy reports referred to in this paper. It was first proposed by Holst and Fröhlich (6), and consists of a more or less arbitrary judgment as to the degree of severity of pathological lesions in teeth, ribs, jaw, intestine, joints, and muscles. Three points indicating the most advanced stages of these lesions—fragility, looseness, or hemorrhage—in each of the eight types of tissue constitute the highest possible scurvy score, which is 24. After much experience the operator may achieve definite skill in allotting the "scurvy score," but at best the method must be looked upon as auxiliary to the use of graded doses in establishing nice distinctions among antiscorbutic minima.

## EXPERIMENTAL RESULTS

### PRUNES

Only the sulphured prunes and apricots retained any part of the antiscorbutic property of the fresh fruit, as had previously been found with peaches. Table 4 shows that of the 14 prune preparations, 6 were somewhat effective in reasonably small doses, 2 of these being frozen fresh fruit (lots 10, 10a), 2 dehydrated sulphured and lye dipped (lots 11, 11a), 1 dehydrated and sulphured but not lye dipped (lot 13), and one sundried, sulphured, and lye dipped (lot 17). The effect of the lye dipping was distinctly favorable to the retention of the vitamin, probably because the surface of the lye-dipped prunes was more easily penetrated by the protecting sulphur dioxide. This supposition is corroborated by the considerably higher sulphur dioxide content of the lye-dipped fruit (lots 11 and 17) as compared with the undipped fruit (lots 13 and 14). The one undipped sample of dehydrated sulphured prunes (lot 13) which retained any trace of antiscorbutic property was distinctly lower in value than the corresponding lye-dipped product (lot 11), but higher than the sundried sample (lot 14) receiving similar treatment. In all cases the sundried samples were lower in antiscorbutic value than the dehydrated ones, even when the amount of sulphur dioxide present was larger in the former.

Some question arises as to the effect of storage upon the sulphur dioxide content of the fruit. Sulphur dioxide in dried fruit disappears during storage (10), although rather slowly. Some discrepancy would doubtless exist, therefore, between the figures given here and the content when drying was completed or when feeding was done. However, the difference would probably be relatively small, since the determinations were made as soon as possible after the samples were placed in storage at 0° C. It is obvious that the protection afforded by the sulphur dioxide is exerted during the actual drying period and that loss of the gas later may be of little importance although not negligible, particularly if the fruit is subjected to higher than usual room temperatures. The resulphuring of dried fruit after washing and before packing, regularly practiced with apricots and peaches and often with apples and pears, can add nothing to vitamin C protection.<sup>4</sup>

Attention is directed to the difference in antiscorbutic property shown by the fresh prunes of the two crops. The 1927 crop with a moisture content of 79.5 per cent showed a distinctly higher vitamin C value than the 1928 crop with moisture content of 62.6 per cent. It is possibly not surprising that the juicier fruit should be richer in the water-soluble antiscorbutic substances. It was found, incidentally, that the vitamin A content of the two fresh prune samples differed in the opposite direction, the more watery 1927 lot being poorer in the vitamin. Again, the fat-soluble vitamin might well be expected to reside in the pulp and to vary with dry content. The fallacy of expressing vitamin C content in terms of dry matter, therefore, is illustrated by the contrast in the values for the fresh prunes shown in Table 4.

#### APRICOTS

The tendency to loss of vitamin C in frozen, sundried, and dehydrated apricots appears to be greater than in peaches or prunes. This tendency to loss of vitamin is even more marked in the case of vitamin A, and points to the probable presence of powerful oxidative catalysts as well as of tissue oxygen in apricots. In comparing the 1928 apricot samples with fresh fruit it was necessary to use frozen fruit of the 1929 crop because of the complete loss of vitamin C in the unevacuated frozen samples of the 1928 crop. The percentage losses given in Table 6 are therefore of only relative value. If the same condition shown by the two prune crops holds for the apricots, however, the more watery 1929 apricots (moisture 85.7 per cent) were slightly richer in vitamin C than the 1928 fruit (moisture 82.9 per cent), and the losses indicated for the dried preparations of the latter crop are probably somewhat too high.

<sup>4</sup> A preliminary abstract of a report given at the Cincinnati meeting, 1930, of the American Chemical Society by E. M. Nelson and D. Breese Jones indicates that processing and resulphuring of apricots as generally practiced results in retention of very little of the antiscorbutic content of the fruit. However, confirmation of the striking preservative effect upon the vitamin of the sulphur dioxide used in the original drying is reported. Tests upon the vitamin C content of resulphured and processed apricots of the 1930 crop are now being made in these laboratories, as well as tests of the effect of cooking upon the same vitamin in the sulphured dried apricots.

TABLE 5.—Vitamin C content of fresh and dried sulphured and unsulphured Royal apicoids

Treatment and crop	Lot No.	SO <sub>2</sub>	Amount fed daily		Average weight of animals			Gain or loss in weight per day	Average length of period	Survival score	Final condition
			Dried fruit	Equivalent in fresh fruit	Animals	Initial	Maximum				
			Grams	Grams	Number	Grams	Grams	Grams	Days		
Fresh frozen, not evacuated, 1928 crop.....	5	P. p. m.	8.0	3.0	2	255	458	295	37	14	Severe scurvy.
			8.0	5.0	4	320	499	247	40	11	Do
			8.0	5.0	6	315	374	244	45	14	Do
			15.0	10.0	4	306	361	242	46	10	Do
			15.0	15.0	4	306	361	263	45	10	Do
Fresh frozen, evacuated before freezing, 1929 crop.....	5a		15-20	20-20	3	351	382	263	71	16	Do
			15.0	15.0	3	347	470	450	71	0	No scurvy.
			20.0	20.0	3	424	460	452	64	20	Do
			20.0	20.0	2	306	436	238	36	22	Severe scurvy
			3.0	4.5	2	306	345	188	45	15	Do
Dehydrated, unsulphured, 1928 crop.....	3	100	5.0	9.0	2	411	363	226	31	19	Do
			10.0	22.6	2	477	440	269	28	13	Do
			15.0	45.3	3	553	361	247	33	13	Do
			15.0	91.5	3	359	401	239	46	16	Do
			15.0	24.3	2	370	390	262	21	15	Do
Dehydrated, sulphured, 1928 crop.....	1	80	15.0	43.3	4	311	447	205	29	15	Do
			1.0	4.7	2	424	376	205	36	14	Do
			4.0	10.1	2	424	376	205	36	14	Do
			15.0	29.1	3	352	380	243	38	18	Do
			15.0	72.5	3	307	343	200	31	12	No scurvy.
Dehydrated, sulphured, 1928 crop.....	1	515	3.0	11.2	3	315	407	211	71	0	No scurvy.
			4.0	17.0	2	326	376	211	90	0	Do
			4.0	28.5	2	364	419	259	60	0	Do
			5.0	24.5	4	370	437	195	78	0	Do
			2.0	12.0	1	381	437	240	38	12	No scurvy.
Dehydrated, sulphured, 1928 crop.....	2a	562	1.0	24.0	3	457	317	217	28	18	No scurvy.
			1.0	24.0	3	410	390	250	42	16	Severe scurvy
			2.0	29.4	1	370	390	250	31	10	Do
			5.0	29.8	2	427	418	288	60	10	Moderate scurvy
			10.0	48.2	4	450	418	290	20	12	Severe scurvy
Sun-dried, sulphured, 1928 crop.....	7	470	4.0	18.9	1	450	509	350	88	1	Severe scurvy
			4.0	28.4	1	440	509	350	88	1	Severe scurvy
			4.0	28.4	1	440	509	350	88	1	Severe scurvy
			4.0	28.4	1	440	509	350	88	1	Severe scurvy
			4.0	28.4	1	440	509	350	88	1	Severe scurvy

An attempt was made with the apricots to find the lower limit of sulphur-dioxide content which would protect the vitamin from destruction during drying. Of the 11 apricot preparations studied (Table 5), 2 were fresh frozen, 2 were sundried, and 7 were dehydrated. Only 2 of the dehydrated samples were unsulphured (lots 3 and 1a), and these were found to be devoid of antiscorbutic value. Of the 5 sulphured dehydrated preparations, 2 were of very low sulphur-dioxide content (lots 2 and 4), and these appeared to be no more valuable than the unsulphured lots. The 3 lots containing larger amounts of sulphur dioxide (lot 1, 515 p. p. m.; lot 2a, 892 p. p. m.; lot 3a, 2,028 p. p. m.) retained their antiscorbutic property satisfactorily. Of the 2 sundried sulphured specimens, lot 6 (700 p. p. m.) compared well with the best of the dehydrated (lot 1), but lot 7 (470 p. p. m.) gave evidence of very low yet demonstrable retention of the vitamin. This figure (450 to 500 p. p. m.) has therefore been taken as representing the border line of effective protection for vitamin C. Lots 2 and 4 had no more sulphur dioxide than did the unsulphured product which represented the blank check on the method of determination of sulphur dioxide, and may therefore be looked upon as practically identical with unsulphured fruit. It must be admitted also that there is disagreement as to the reliability of methods of determining sulphur dioxide in fruit, and that consequently fine distinctions can hardly be drawn at present on this point. However, the vitamin C values of these apricot samples accord remarkably well with the theory that the sulphur-dioxide content must reach a certain minimum in order to be effective in protecting the vitamin, and likewise that sulphuring beyond this point usually adds little to the protection. (Compare lots 2a and 3a.) However, the difference in vitamin retention between prune lots 11 (1,980 p. p. m.) and 11a (887 p. p. m.) may be cited as indicating some effectiveness beyond the minimum.

A recent report by Williams and Corran (13) indicates the failure of potassium metabisulphite as preservative of vitamin C of lemon juice but success in the case of lemon juice kept in a sulphured cask. These results seem to indicate an injurious action by one of the substances, other than sulphur dioxide, introduced in the gradual decomposition of the metabisulphite, or else an insufficient release of sulphur dioxide. Some means of adding the sulphur dioxide to fruit juices, other than by use of metabisulphites, seems indicated.

In comparing the percentage retention of vitamin C in the fresh fruit with that in the various dried products as shown in Table 6, the same assumptions were made as in the study of vitamin A previously reported (9). The minimum protective dose of the fresh fruit was used as the basis and was compared with the minimum protective dose of the dried product, calculated in equivalent of fresh fruit. The calculation is arbitrary and must be recognized as affording only approximately accurate comparisons.

TABLE 6.—*Summary of experimental results on vitamin C retention in dried peaches, prunes, and apricots, sulphured and unsulphured*

Fruit	Method of preparation and crop	Lot No.	Minimum daily dose required to protect standard guinea pig against scurvy (equivalent in fresh fruit)			Average daily gain of weight of animals	Vitamin C retention
			Grams	Grams	Per cent		
Peaches (89)	Fresh, frozen.	F	5-8	1.1			100
	Dehydrated, sulphured	SE	1	1.7			100
	Sundried, sulphured	SD	4	.8			100
	All unsulphured	D, E	40+	(b)			0
	Fresh, frozen, 1927 crop	10a	12	2.1			100
	Fresh, frozen, 1928 crop	10	10-20	1.0			100
Prunes	Dehydrated and sundried, sulphured, lye dipped, 1928 crop.	11, 17	11-18	1.6			100
	Dehydrated, sulphured, lye dipped, 1927 crop	11a	20	7			60
	Dehydrated, sulphured, not lye dipped, 1928 crop	13	33+	.1			50-
	Sundried, sulphured, not lye dipped, 1928 crop.	14	32+	(b)			0
	All unsulphured, 1927 and 1928 crops	12, 12a, 15, 18, 18a, 19, 16	41+	(b)			0
	Fresh, frozen, not evacuated, 1928 crop	5	30+	(b)			0
Apricots	Fresh frozen, evacuated, 1929 crop	5a	15	2.2			100
		1	17	2.1			d 100
		2a	21	2.5			62
		3a	29	2.7			52
		6	28	1.5			c 52
		7	38+	1.0			39--
				1.0			
		1a	81+	(b)			0
		2	72+	(b)			0
		3	15+	(b)			0
		4	73+	(b)			0

<sup>a</sup> + indicates that more than the weight given, and which was tried, would be necessary for protection

<sup>b</sup> Severe scurvy.

<sup>c</sup> - indicates that less than the percentage given was retained.

<sup>d</sup> All products dried in 1928 compared on basis of 1929 fresh apricot vitamin content; these two results are approximate.

<sup>e</sup> Mild scurvy.

<sup>f</sup> Normal.

## SUMMARY

The vitamin C content of frozen fresh prunes and apricots and of prunes and apricots dried by various methods was determined by biological technic.

Doses of the different fruit products were fed to guinea pigs for 60 and 90 days, and the rate of growth of the animals receiving the various doses was compared. Examinations were also made for symptoms of scurvy, and in cases where animals died because of insufficient protection from scurvy the length of the survival period was determined. The 60-day period is shown to be as effective for assay of vitamin C as the 90-day period.

Frozen fresh prunes of two crops retained the vitamin C satisfactorily, but frozen fresh apricots packed in cases which were not evacuated, lost all of this property. A second lot packed in cases which were evacuated and filled with nitrogen before the fruit was frozen, retained the vitamin. The difference is ascribed to retention of tissue respiratory oxygen in the unevacuated lot.

Sulphured, dehydrated, and sundried prune products retained the vitamin C of the fresh fruit satisfactorily only when the fruit was dipped in lye in the usual commercial fashion before the sulphur

dioxide treatment. This is ascribed to better penetration by the protecting sulphur dioxide after the lye dipping.

All unsulphured products of both fruits whether sundried or dehydrated were without antiscorbutic value.

The dehydrated products both prune and apricot, retained the vitamin C more completely than did the corresponding sundried fruit.

The dehydrated and sundried apricots containing 450 to 500 or more parts of sulphur dioxide per million retained the antiscorbutic property more or less completely. With less than this amount, all products lost this property completely.

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# A METHOD FOR REMOVING FAT SAMPLES FROM LIVE HOGS<sup>1</sup>

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## INTRODUCTION

In studying variations in the properties of fat as the fattening process advances it is often advisable to take successive samples from live animals. An experiment at the Purdue University Agricultural Experiment Station, conducted for the purpose of determining the influence of the degree of fatness of hogs on the quality of pork, provided the incentive for devising the sampling method described in this paper.

In early studies of fat development investigators used the method of killing "representative" individuals at different stages of development. In recent years workers have come to recognize the importance of the individuality factor. Scott<sup>3</sup> in 1920 emphasized specifically the great variation in the properties of the fat from different individuals kept on the same ration. Hankins and Ellis,<sup>4</sup> using the method of killing representative animals, also recognized individual variation and attempted to overcome its effect by using three animals at each killing.

Ewing and his coworkers<sup>5</sup> in 1919 described a method of removing fat samples from live hogs by means of a twisted clock-spring bit fitting inside a cannula. Later Scott<sup>3</sup> reported work in which he had removed fat samples from the rear part of the ham by making a slit about 2 inches long and taking out a piece of fat. A modification of this process in which the fat was removed through an incision in the back has been used recently at the University of Illinois.<sup>6</sup>

In agreement with the early work of Henriques and Hansen<sup>7</sup> it was found at the Purdue Agricultural Experiment Station<sup>8</sup> that the different layers of the adipose tissue of hogs yield fat of different constants. It was realized therefore that special precautions should be taken to insure proportionate amounts of the different layers in the sample when developmental studies are being made. This factor was considered of such importance as to warrant a careful study of the sampling process. It was felt that a sampling method should meet the following requirements: (1) Samples showing development of fat should come from the same individual; (2) they should be large

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<sup>2</sup> Appreciation is expressed to Dr. F. P. Mathews of the Veterinary Department of Purdue University for his helpful suggestions in connection with the technique of the operation.

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enough for the necessary chemical determinations; (3) they should be uniform in width and should extend clear through the fat tissue; (4) they should be taken from the same region, or from fat tissue of similar properties; (5) the sampling should be done in such a way as to cause the animal as little discomfort and setback as possible; (6) the incision should be made in a place where the chance for infection is slightest, and in such a way that the resulting wound will not be subjected to excessive strain; (7) the sample removed should not contain injected materials.

### PRELIMINARY WORK

That the variation in the properties of the fat from samples taken at different locations on the fat backs of slaughtered hogs is so small as to be insignificant was the conclusion reached as a result of preliminary work at this station.<sup>9</sup> It was therefore considered that samples properly taken from the part of the back corresponding to the fat back would give comparable results.

At the beginning of an experiment conducted at Purdue University 80 pigs were sampled and the greater number of these were again sampled at intervals of 30 days for four successive samplings. Because of the results obtained in this and other experiments it is believed that the method herein described meets all of the requirements set forth above.

### THE METHOD

#### PREPARING THE HOG

The hog is confined in a restraining crate of the hyperimmunization type, which is so designed as to hold him firmly in position. The top of the crate is open to permit the operator access to the back of the hog. A spot about the size of a man's hand directly over the loin is shaved, washed, and disinfected. By means of mercurochrome an oblong about 6 inches long and 2 inches wide is drawn on the shaved skin.

#### INJECTING THE ANAESTHETIC

With a metal hypodermic syringe the anaesthetic is injected intradermally along the mercurochrome line. The needle is inserted between the layers of the skin and is gradually pushed in to its full length while small amounts of the anaesthetic are being injected. When the entire skin oblong has been deadened a small quantity of the anaesthetic is injected between the fat tissue and the muscular layer at each end and side of the oblong and this completes the anaesthesia.

#### REMOVING THE FAT SAMPLE

When the skin is no longer sensitive the incision is made. An oblong piece of skin and fat about 4 or 5 inches long and thick enough to provide an adequate sample is removed. In making the incision it is advisable to keep well within the swellings caused by the injected liquid. Figure 1 illustrates the sampling method, gives a diagram of the sample removed, and shows the method of closing the wound. Care should be taken that the entire fat tissue is sectioned clear through to the muscular layer, and that the incision is so made as

<sup>9</sup> Block, H. W. Op. cit.

to insure uniform width of the fat layers. If the layer of fat is thick the strip of tissue removed may be comparatively narrow, affording sufficient sample and yet permitting the wound to be closed easily.

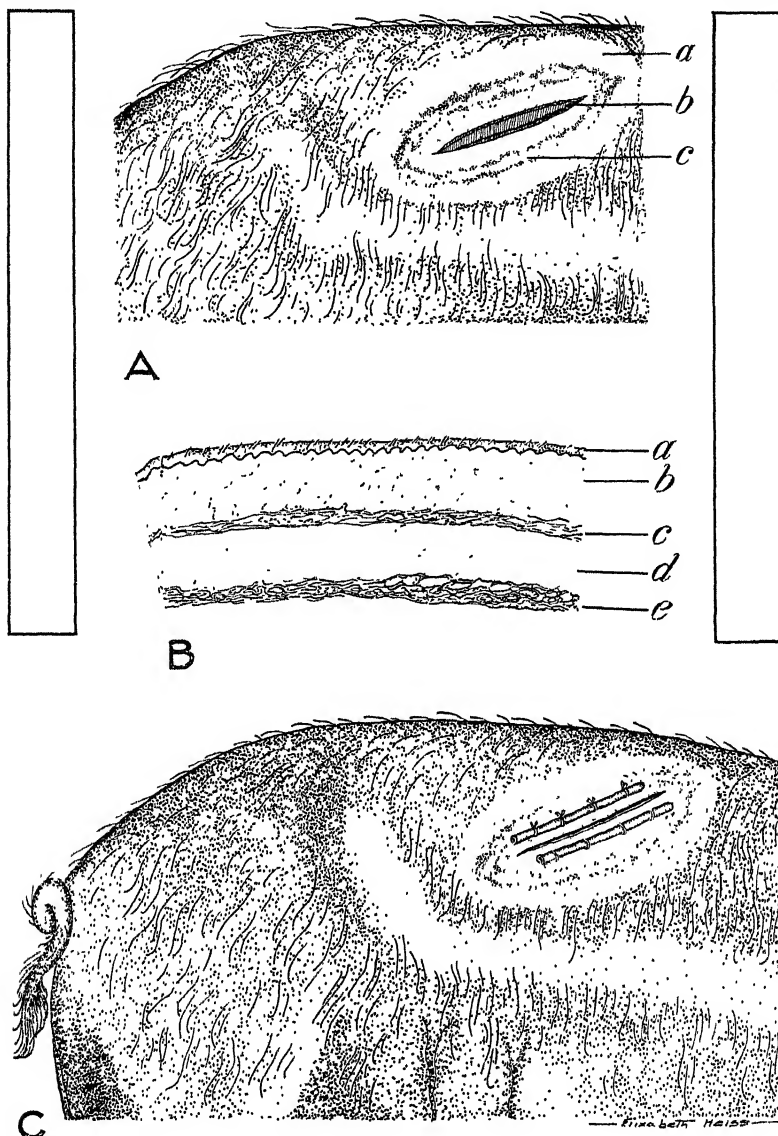


FIGURE 1.—Method of removing fat samples from live hogs. A, Incision for removing sample. a, Shaved area; b, incision; c, swellings where anaesthetic was injected. B, Diagram of removed tissue. a, Skin; b, subcutaneous fat layer; c, connective tissue; d, inner fat layer; e, connective tissue and blood vessels. C, Method of closing wound.

#### CLOSING THE WOUND

By means of four quilled sutures, using half-curved cutting-edge suture needles and aseptic machinist's thread, the wound is closed. A thick-walled,  $\frac{3}{16}$ -inch rubber tubing on each side of the wound

proved effective in providing anchorage and in preventing the stitches from cutting through the skin. The wound is disinfected with 50 per cent alcohol and is then covered with pine tar. A little fish oil mixed in the tar renders the antiseptic dressing fly repellent.

#### HANDLING THE SAMPLE

After the sample has been removed it should be blotted free of blood, the skin taken off, and the network of blood vessels and connective tissue dissected from its ventral surface. Samples should be promptly placed in refrigeration and kept there until they can be delivered to the chemistry laboratory.

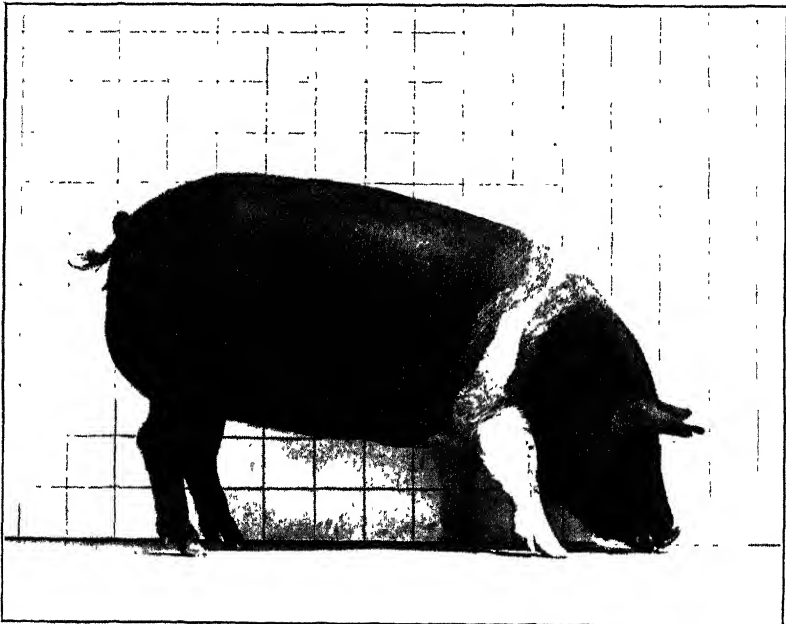


FIGURE 2.--An experimental hog from which four fat samples have been removed

#### ADDITIONAL OBSERVATIONS

Sterilization of instruments and materials was accomplished by boiling them in water. Fifty per cent alcohol proved to be a satisfactory antiseptic. An occasional case of infection occurred, but the hog invariably recovered.

Several different anaesthetics were tried. With magnesium sulphate the writers were unable to get proper anaesthesia. Administration of novocaine caused swelling and the animal would not resume feeding for about two days. The use of benzyl alcohol caused sloughing of the tissues. Finally a 1 per cent solution of apothesine (the hydrochloride of  $\gamma$ -diethylaminopropylcinnamate) was adopted as a convenient, efficient, and inexpensive anaesthetic.

The effectiveness of the anaesthetic depends upon the success of the operator in injecting intradermally. Black hogs (Poland Chinas and Hampshires) have a much thicker and less pliable skin than do

Yorkshires, for example. The latter are more difficult to anaesthetize, but skill in injecting the solution overcomes the difficulty.

Samples from the experimental hogs were taken adjacent to but on opposite sides of the backbone. In the early part of the experiment the incisions were made parallel to the backbone. Later it was found that if the incisions were made on the back running posteriorly and ventrally at an angle of approximately  $45^\circ$  to the backbone, good drainage was afforded and the healing was somewhat facilitated.

In the course of 8 to 10 days the wound appears to be healed, and most of the threads will give way and fall off with the rubber tubing. An individual from which four samples have been removed and the wounds healed is shown in Figure 2.

With one attendant to prepare the hogs, one operator to administer the anaesthetic and care for the samples, and another to remove the samples and dress the wounds, about 25 or 30 hogs can be sampled in eight hours.

This method has given good results with swine. With slight modifications it may be used in studying progressive fat development in other classes of meat animals.

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# A NEGATIVE CORRELATION OBSERVED BETWEEN THE NITRATE NITROGEN IN THE JUICE OF BEET LEAVES AND THE WEIGHT OF THE LEAVES<sup>1</sup>

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## INTRODUCTION

Several workers have reported correlations between age or size of leaf and the physical or chemical properties of the leaf juice. Chandler (1),<sup>2</sup> working with apples and peaches, found the juice of the younger leaves to be less concentrated than that of older leaves, and Dixon and Atkins (4) found the same to be true of lilacs and other plants. Pringsheim (11), on the other hand, reported that the old leaves of *Coleus* yield a less concentrated juice than the younger leaves. Working with cornstalks, Hurd-Karrer (9) found the juice of the internodal sections to have a higher specific gravity nearer the tip of the plant. Eaton (5) noted that the juice from the older leaves of the cotton plant had a greater freezing-point depression than that from the young leaves. Chibnall (2) has attempted to compensate for the different sizes of leaves when analyzing plant tissue by segregating the leaves of different sizes and taking a representative proportionate aliquot weight.

During a series of determinations of nitrate nitrogen in various vegetable juices, the writer observed that the fluctuations in the quantity of this constituent were considerably larger than could be accounted for by the experimental error of the method. In the course of further investigation, a casual examination showed a significant negative correlation between the weight of beet leaves and the amount of nitrate nitrogen in the juice of these leaves. (Table 1.) In order to determine the significance of this observation, in the light of the results of the workers mentioned above, and to find an explanation for the phenomenon, the investigation herein described was carried out.

TABLE 1.—*Nitrate nitrogen in juice obtained from different weights of beet leaves*

Leaves	Average weight per leaf	Nitrate nitrogen in juice
<i>Number</i>	<i>Grams</i>	<i>Parts per million</i>
30	0.86	73
30	1.83	52
30	3.73	40
30	8.86	15

## EXPERIMENTAL DATA

The beets considered in this paper were grown on  $\frac{1}{80}$ -acre market-garden plots at the Rhode Island Agricultural Experiment Station. These plots and their treatments have been described in detail by Hartwell and Crandall (7), and further discussed by Smith (12).

<sup>1</sup> Received for publication June 23, 1930, issued January, 1931. Published by permission of the director of the Rhode Island Agricultural Experiment Station as contribution No. 397.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 56.

The beets were planted April 13, 1928. The leaves used were taken on July 28 from plants growing on plots fertilized with an optimum amount of nitrogen, and were nearly mature.

Leaves were taken at random over the entire area of the plot from a number of individual plants estimated to be sufficient to obviate significant sampling errors. As soon as the leaves were picked they were taken to the laboratory, weighed, and the nitrate nitrogen in the juice was determined by the method of Gilbert (6). The data which exhibit the correlation are shown in Table 2.

TABLE 2.—*Correlation between nitrate nitrogen in beet juice and weight of leaves yielding juice*

Leaves	Average weight per leaf	Nitrate nitrogen in juice	Leaves	Average weight per leaf	Nitrate nitrogen in juice
Number	Grams	Parts per million	Number	Grams	Parts per million
10	6.5	9.8	40	4.7	42.5
20	5.7	13.8	49	3.7	35.9
20	5.9	27.6	50	4.3	31.1
29	4.0	42.5	60	3.8	31.8
30	4.6	42.0	115	3.2	37.6
40	4.2	34.7	115	3.2	70.4

Coefficient of correlation  $r = -0.855 \pm 0.052$ .

In calculating the coefficient of correlation the following formula was used:

$$r_{XY} = \frac{\frac{\sum XY}{n} - \bar{X}\bar{Y}}{\sqrt{\frac{\sum (X^2)}{n} - \left[\frac{\sum (X)}{n}\right]^2} \sqrt{\frac{\sum (Y^2)}{n} - \left[\frac{\sum (Y)}{n}\right]^2}}$$

This formula, discussed by Hayes and Garber (8, p. 43), is especially applicable to small numbers of individuals. The probable error was calculated by the formula;

$$P.E._r = 0.6745 \times \frac{(1-r^2)}{\sqrt{n}}.$$

The fact that the juice expressed from the midrib of a leaf is usually different in chemical composition from that expressed from the more actively metabolizing mesophyll tissue is well known to investigators. Few, however, have discussed this matter, preferring to eliminate an obvious source of error by removing the midribs before making the analysis. Among the many workers who have followed this procedure, Chibnall (2, 3), Maskell and Mason (10), and Gilbert (6) may be mentioned.

It was learned early in the course of the investigation of the nitrate concentration in the juices of crop plants that the juice of the midribs of most leaves have a much higher concentration of this constituent than the juice from the remainder of the leaf. The data for beet leaves are given in Table 3.

TABLE 3.—*Nitrate nitrogen in fluids expressed from the midribs of beet leaves as compared with that from the remainder of the leaves*

[In parts per million]	
Nitrate nitrogen in midribs	Nitrate nitrogen in leaves minus midribs
175	75
2,500	327
160	10
188	16
280	19

These figures suggest that the ratio of midrib tissue to the remainder of the leaf tissue varies with the size of the leaf, and if such is the case the relative amounts of nitrates present in the juice from large and small leaves would also vary. To determine the relation between the weight of the midrib and the weight of the remainder of the leaf both large and small beet leaves were gathered, the midribs were dissected out with a scalpel, and the two separate fractions weighed. Table 4 gives the results of these determinations.

TABLE 4.—*Relation of weight of midribs to weight of remainder of leaves for large and small beet leaves*

Leaves	Average length of leaves	Weight of leaves minus midribs	Weight of midribs	Weight of leaves minus weight of midribs Weight of midribs
Number	Cms	Grams	Grams	
15 <sup>a</sup>	22	155.00	27.50	5.64
15 <sup>b</sup>	11	24.91	6.25	3.98
20 <sup>a</sup>	18	185.70	39.10	4.75
20 <sup>b</sup>	8	17.82	4.72	3.77

<sup>a</sup> Large.

<sup>b</sup> Small.

The differences in the ratio of the weight of the remainder of the leaf tissue to the weight of midrib tissue are comparatively large, and their magnitude is great enough, in the writer's estimation, to offset any crudity in the methods.

The negative correlation between the weight of leaf tissue and the nitrate concentration in the juice from these leaves is satisfactorily explained by the following facts:

(1) The juice of the midrib of the beet leaf contains considerably larger quantities of nitrate than does the juice from the remainder of the leaf.

(2) The ratio by weight between the leaf tissue less midrib to the midrib tissue is higher in large leaves, indicating a smaller proportion of midrib in these leaves.

It is obvious that when the tissue containing the greater quantity of nitrates is present in considerably smaller proportion of the whole leaf, the nitrate concentration in the juice of the leaf as a whole will be considerably less, thus accounting for the negative correlation observed.

It follows from the above explanation that in order to reduce the possibility of serious error in the sampling of comparative areas



of beets, it is necessary to choose leaves that are approximately the same size. It has been found that removal of the midrib tissue before analysis is necessary to obtain concordant results, since such high concentrations of nitrate nitrogen as exist in the juice of this tissue may introduce serious error.

This work is concerned only with the nitrate-nitrogen content of the juice of beet leaves. There is little doubt, however, that other constituents of the juice of beets, and perhaps of other plants, vary considerably with the weight of the leaf.

#### SUMMARY

A negative correlation was observed between the nitrate nitrogen in the juice of beet leaves and the weight of the leaves yielding the juice. It was also observed that juice from the midrib of the beet leaf contains considerably larger quantities of nitrate than does that from the remainder of the leaf. Finally, there is a smaller proportion of midrib by weight in large than in small leaves. Hence it is necessary to choose leaves of approximately the same size for analysis in order to avoid the possibility of a serious sampling error.

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## FREEZING-POINT DEPRESSION AND SPECIFIC CONDUCTIVITY OF SORGHUM TISSUE FLUIDS<sup>1</sup>

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### INTRODUCTION

The sorghums probably are the most outstandingly drought resistant of American cultivated crops. Their ability to survive periods of moisture deficiency with little injury has long been recognized. The sorghums include extremely diverse groups within a single species. One group, the sorgos or saccharine sorghums, produces an abundance of sugar which is stored in the juice of the stalks. Grain sorghums, including kafir, milo, feterita, durra, kaoliang, and hegari, tend to produce larger quantities of seed, and more of the carbohydrates are thus diverted to starch production. Broomcorn, on the other hand, produces numerous elongated panicle branches which form the fibrous brush. The groups and varieties of sorghum show wide variations in growth habits and in disease and insect resistance. The term "sorghums" as used in this paper includes both grain sorghums and sorgos and, in some cases, broomcorn also.

The present studies were undertaken to determine differences that might exist in tissue-fluid properties of sorghum varieties under different conditions and the changes that take place in these properties. One or two varieties of corn were grown each season for comparison of their tissue-fluid properties with those of the sorghums.

### EXPERIMENTAL METHODS

The experiments here reported were limited largely to determinations of the freezing-point depression and the conductivity of the expressed plant juices. In 1926, however, some determinations of total solids and "bound water" were made. Freezing-point depressions were determined by means of ordinary Beckman apparatus and a Heidenhain thermometer, and the conductivity was measured by the Wheatstone bridge at a temperature of 30° C. Total solids were measured with the Abbe refractometer, and bound water by the method of Newton and Gortner.<sup>4</sup>

The sorghums were grown under irrigation and received water whenever necessary to maintain growth, except in some experiments

<sup>1</sup> Received for publication Nov. 20, 1930; issued January, 1931.

<sup>2</sup> The final draft of this manuscript was not completed until after Doctor Harris's death.

<sup>3</sup> The writers wish to acknowledge the assistance of C. J. King, superintendent of United States Field Station, Sacaton, Ariz., in planting and growing the crop, and of C. W. Crane, T. A. Pascoe, J. P. Barton, D. R. Briggs, and J. Arthur Harris, Jr., in collecting samples and making the determinations.

<sup>4</sup> NEWTON, R., and GORTNER, R. A. A METHOD FOR ESTIMATING HYDROPHILIC COLLOID CONTENT OF EXPRESSED TISSUE FLUIDS. *Bot. Gaz.* 74: 442-446, illus. 1922.

in which irrigation was withheld purposely. Samples of the leaves or stalks were gathered just about sunset, stuffed into tubes, and the tubes placed in a salt-and-ice mixture for freezing. After freezing for 24 hours or more, the samples were thawed and pressed. The extracted juices were kept cold until the determinations were made. All samplings and determinations were made in duplicate and the results averaged.

When leaves from different positions on the plant were being studied, samples were taken from several plants. For other studies all or most of the leaves from a single plant were kept together. The midribs were removed from the leaf samples. The internode samples consisted of the pith with the cortical layers peeled off. They were also composites of all or most of the internodes from a single plant or of single internodes from different plants.

The experiments herein reported were conducted at the United States Field Station, Sacaton, Ariz., during the late summers of four successive years from 1926 to 1929. The results presented, while not final in all cases, are believed to be sufficiently reliable to indicate general characteristics.

## RESULTS OBTAINED

### LEAVES AND INTERNODES

The values for the freezing-point depression of the juices of leaves and internodes in different positions on the stalk are shown in Table 1. Data are presented for seven varieties of sorghum sampled on three dates during a period of about a month. The first sampling was made about heading time for most of the varieties, the second after the completion of blooming, and the third when the seeds were in the dough-to-ripe stage. The positions of both leaves and internodes are indicated by numbers, beginning with 1 for the upper or terminal leaf or internode and increasing downward.

The upper internode, or peduncle, protrudes from the sheath of the upper leaf and is attached at the upper node, to which the upper leaf sheath also is attached. The upper terminal leaf is the smallest in size, the second is considerably larger, while the third is the largest leaf on the stalk. Below the third leaf the size decreases gradually. The oldest leaves and internodes are nearest the base of the stalk. As the plant approaches maturity the leaves, beginning with the lower ones, gradually become dry and may break off the stalk.

The average results in Table 1 show that the freezing-point depression is greatest in the juices of the upper or terminal leaf, with a gradual decrease from the top downward. This occurred at all three periods of sampling. The concentration of the juice of all leaves was only slightly greater in the third sampling, when the seeds were nearly mature, than in the first sampling, when most of the heads were in bloom.

The freezing-point depression of the stalk juice in the various internodes was practically the same on each of the first two dates, although the concentration in all internodes had increased in the interval between the two samplings. The concentration of the juice of all internodes except the first had increased still more by the third sampling date.

By the third sampling date, when the plants were nearly mature, a variation in the juice concentration in the different internodes also was apparent, increasing from the first down to the fourth internode, with the fifth and sixth internodes about the same as the fourth.

TABLE 1.—*Freezing-point depression of the juices of leaves and internodes on three dates, Sacaton, Ariz., 1926*

	Freezing-point depression (° C.) of—							
Juices, date of sampling, and leaf or internode No. <sup>a</sup>	Dwarf Yellow milo	Dawn kafir	Reed kafir	Dwarf hegmi	Standard fet-eita	Black Amber sorgo	Kansas Orange sorgo	Average
Leaf juices:								
July 28 or 30, from leaf No.—								
1.....	1.04	0.96	0.79	1.31	1.10	0.93	0.94	1.01
2.....	.99	.88	.92	1.06	1.03	.95	.97	.97
3.....	.98	.86	.93	.93	.98	.89	1.06	.95
4.....	<sup>b</sup> 1.00	.83	.94	.87	.92	.83	1.07	.92
5.....	1.03	.81	.95	.82	.86	.82	.98	.90
6.....	.92	.78	.91	.82	.86	.81	.93	.86
7.....				.94				
Aug. 5 or 9, from leaf No.—								
1.....		.92		.91	1.08	.86		<sup>c</sup> .94
2.....	1.04	.85		.92	1.06	.88		<sup>c</sup> .93
3.....	1.05	.84		.80	.97	.81		<sup>c</sup> .86
4.....	1.02	.82		.84	.93	.80		<sup>c</sup> .85
5.....	.98	.81		.77	.90	.78		<sup>c</sup> .82
6.....	.97	.78		.81	.85	.76		<sup>c</sup> .80
7.....	.98	.77		.73	.82	.76		<sup>c</sup> .77
8.....	.99	.70			.77			
9.....	.96	.81			.78			
Aug. 28 to Sept. 1, from leaf No.—								
1.....	.94	1.00	1.04	1.07	1.06	1.17	1.13	1.06
2.....	.95	.91	.99	1.00	1.02	1.15	1.16	1.03
3.....	.92	.87	.95	.92	.94	1.08	1.17	.98
4.....	.92	.88	.92	.90	.94	1.03	1.18	.97
5.....	.90	.86	.88	.88	.90	.98	1.15	.94
6.....	.91	.81	.82	.87	.90	.96	1.13	.91
7.....		.81	.77		.92	.90	1.11	
8.....							1.09	
9.....							1.05	
Internode juices:								
July 28 or 29, from internode No.—								
1.....	.95	1.03		<sup>d</sup> 1.11	1.24	1.14		1.09
2.....	1.04	1.01		<sup>d</sup> 1.20	1.28	1.08		1.12
3.....	.96	.98		<sup>d</sup> 1.23	1.28	1.05		1.10
4.....	1.14	.99		<sup>d</sup> 1.24	1.28	1.08		1.15
5.....	.99	.91		<sup>d</sup> 1.25	1.28	1.03		1.09
6.....	1.11	.88		<sup>d</sup> 1.27	1.28	1.04		1.12
7.....				<sup>d</sup> 1.29				
8.....				<sup>d</sup> 1.24				
9.....				<sup>d</sup> 1.30				
Aug. 5 or 9, internode No.—								
1.....	1.32	1.38			1.37	1.33		1.35
2.....	1.19	1.33			1.36	1.26		1.29
3.....	1.29	1.35			1.34	1.21		1.30
4.....	1.27	1.38			1.22	1.26		1.28
5.....	1.22	1.34			1.28	1.16		1.25
6.....	1.27	1.29			1.29	1.28		1.28
7.....	1.21	1.27			1.26	1.11		1.21
8.....	1.24	1.27			1.35	1.16		1.26
9.....	1.20	1.27			1.45	1.20		1.28
Aug. 28 to Sept. 1, from internode No.—								
1.....	1.30	1.39	1.30	1.29	1.45	1.35	1.57	<sup>e</sup> 1.36
2.....	1.50	1.65	1.46	1.61	1.59	1.47	1.77	<sup>e</sup> 1.56
3.....	1.51	1.69	1.47	1.64	1.73	1.56	1.84	<sup>e</sup> 1.63
4.....	1.54	1.79	1.52	1.79	1.85	1.70	1.91	<sup>e</sup> 1.73
5.....	1.55	1.62	1.49	1.74	1.88	1.67	1.92	<sup>e</sup> 1.69
6.....	1.45	1.60	1.44	1.87	1.89	1.65	1.86	<sup>e</sup> 1.69
7.....			1.44		1.86	1.63	1.86	
8.....							1.77	
9.....							1.70	

<sup>a</sup> Beginning with terminal leaf or internode.

<sup>b</sup> Interpolated from leaves 3 and 5.

<sup>c</sup> Not including Dwarf Yellow milo.

<sup>d</sup> Aug. 5, Dwarf hegari was late.

<sup>e</sup> Not including Reed kafir and Kansas Orange sorgo.

The average specific conductivity of the juices from leaves in different positions, shown in Table 2, was nearly the same at all three samplings, although the conductivity of the lowest leaves was slightly less than the others. The average conductivity of the juices from the third sampling of leaves was slightly higher than that of the first sampling.

TABLE 2.—*Specific conductivity of the juices of leaves and internodes at three dates, Sacaton, Ariz., 1926*

	Specific conductivity (reciprocal ohms) of—							
Juices, date of sampling, and leaf or internode No. <sup>a</sup>	Dwarf Yellow milo	Dawn kafir	Reed kafir	Dwarf hegari	Standard foterita	Black Amber sorgo	Kansas Orange sorgo	Average
Leaf juices:								
July 28 or 30, from leaf No.—								
1.....	0.0107	0.0164	0.0120	0.0207	0.0122	0.0120	0.147	<sup>b</sup> 0.0147
2.....	.0130	.0153	.0150	.0182	.0126	.0131		<sup>b</sup> 0.0148
3.....	.0139	.0161	.0161	.0169	.0127	.0128	.0114	<sup>b</sup> 0.0149
4.....		.0147	.0170	.0165	.0126	.0119	.0148	<sup>b</sup> 0.0145
5.....	.0150	.0150	.0176	.0161	.0125	.0115	.0159	<sup>b</sup> 0.0145
6.....	.0139	.0130	.0172	.0148	.0128	.0111	.0166	<sup>b</sup> 0.0138
August 5 or 9, from leaf No.—								
1.....		.0156		.0159	.0140			<sup>c</sup> 0.0155
2.....	.0135	.0151		.0161	.0148	.0159		<sup>c</sup> 0.0157
3.....	.0162	.0157		.0159	.0146	.0165		<sup>c</sup> 0.0153
4.....	.0166	.0111		.0178	.0157	.0161		<sup>c</sup> 0.0153
5.....	.0156	.0155		.0155	.0146	.0155		<sup>c</sup> 0.0153
6.....	.0154	.0141		.0158	.0149	.0152		<sup>c</sup> 0.0150
7.....	.0140	.0144		.0144	.0147	.0114		<sup>c</sup> 0.0137
8.....	.0114	.0107			.0153			
9.....		.0132			.0141			
August 28 to September 1, from leaf No.—								
1.....		.0162	.0180	.0179	.0153	.0166	.0159	<sup>b</sup> 0.0168
2.....	.0156	.0169	.0187	.0182	.0159	.0171	.0183	<sup>b</sup> 0.0174
3.....	.0161	.0167	.0190	.0166	.0161	.0179	.0197	<sup>b</sup> 0.0173
4.....	.0174	.0172	.0182	.0170	.0161	.0170	.0200	<sup>b</sup> 0.0171
5.....	.0164	.0170	.0177	.0166	.0151	.0164	.0181	<sup>b</sup> 0.0166
6.....	.0171	.0161	.0168	.0152	.0151	.0155	.0201	<sup>b</sup> 0.0157
7.....		.0161	.0156			.0140	.0184	
8.....							.0202	
9.....							.0191	
Internode juices.								
July 28 or 30, from internode No.—								
1.....	.0066	.0092		<sup>d</sup> .0077	.0162	.0085		<sup>c</sup> .0080
2.....	.0101	.0083		<sup>d</sup> .0113	.0191	.0068		<sup>c</sup> .0091
3.....	.0085	.0101		<sup>d</sup> .0131	.0172	.0061		<sup>c</sup> .0095
4.....	.0141	.0090		<sup>d</sup> .0135	.0172	.0072		<sup>c</sup> .0112
5.....	.0151	.0104		<sup>d</sup> .0138	.0148	.0062		<sup>c</sup> .0114
6.....	.0176	.0124		<sup>d</sup> .0135	.0153	.0087		<sup>c</sup> .0131
7.....				<sup>d</sup> .0139				
8.....				<sup>d</sup> .0164				
9.....				<sup>d</sup> .0182				
August 5 or 9, from internode No.—								
1.....	.0109	.0132			.0173	.0098		.0128
2.....	.0135	.0092			.0161	.0070		.0115
3.....	.0198	.0119			.0183	.0080		.0145
4.....	.0249	.0128			.0138	.0066		.0145
5.....	.0243	.0123			.0139	.0075		.0145
6.....	.0225	.0126			.0144	.0065		.0140
7.....	.0247	.0155			.0141	.0124		.0167
8.....	.0240	.0198			.0156	.0126		.0180
9.....	.0237	.0191			.0171	.0141		.0185
August 28 to September 1, from internode No.—								
1.....	.0248	.0174	.0171	.0245		.0199	.0127	<sup>c</sup> .0217
2.....	.0247	.0141	.0162	.0263	.0282	.0130	.0083	<sup>c</sup> .0195
3.....	.0280	.0151	.0193	.0228	.0187	.0107	.0092	<sup>c</sup> .0192
4.....	.0317	.0144	.0210	.0195	.0163	.0091	.0089	<sup>c</sup> .0187
5.....	.0321	.0140	.0192	.0202	.0151	.0090	.0087	<sup>c</sup> .0188
6.....	.0290	.0136	.0182	.0180	.0151	.0096	.0075	<sup>c</sup> .0176
7.....		.0140	.0167		.0149	.0094	.0079	
8.....							.0086	
9.....							.0078	

<sup>a</sup> Beginning with terminal leaf or internode.

<sup>b</sup> Not including Dwarf Yellow milo and Kansas Orange sorgo.

<sup>c</sup> Not including Dwarf Yellow milo.

<sup>d</sup> Aug. 5, Dwarf hegari was late.

<sup>e</sup> Average of Dwarf Yellow milo, Dawn kafir, Dwarf hegari, and Black Amber sorgo.

The juices of the various internodes show decided differences in conductivity. In the first sampling the average conductivity increased rapidly from the upper internode downward, and a similar but somewhat irregular tendency prevailed at the second sampling. This situation was reversed, however, in the last sampling, as the upper internodes showed the highest conductivity. The average conductivity of the juice of all internodes was much higher in the final sampling than in the first sampling.

It is evident from the above results that food materials do not accumulate in the leaf juices of sorghum plants at maturity. Collier<sup>5</sup> obtained a decrease in sugars in sorgho leaf juices, an increase in "solids not sugar," and a slight increase in the specific gravity of leaf juices between blooming and maturity.

The internodes of sorghums serve as storage reservoirs for sugars. As the plant approaches maturity after the flowering period, sugars increase in the internodes, the greatest accumulation being in the fourth and lower internodes. The salts, on the other hand, accumulate to the greatest extent in the upper internodes with a gradual decrease downward in the stalk. The higher conductivity in the upper internodes probably is partly due to earlier drying in this region as the plants approach maturity.

The concentration gradient in sorghum stalks, as measured by the freezing-point depression, did not correspond to the falling gradient from the top downward established for cornstalks by Hurd-Karrer<sup>6</sup> through specific-gravity measurements of internode juices. The freezing-point depression of the leaf juices at all stages and the conductivity of the sorghum internode juices at maturity, however, showed the same tendency toward a decrease from the top down as reported for corn.

Collier<sup>7</sup> showed that the lower and middle portions of stalks of sorgho, corn, and sugarcane contained more juice and sugars than the top portions. The juices in the top portion of sugarcane stalks contained more salts or ash than those in the middle and butt. The juice from the tops of sorghum stalks contained more "solids not sugar" (chiefly ash and inorganic acids) than that from the middles and butts. The specific gravity and the total-solids content of the juices were nearly constant in different portions of the stalk of corn and sorghums. In sugarcane, however, the juices of the tops were lower than those of the middles and butts in both specific gravity and solids. Collier's results indicated that salts accumulate mostly in the upper portion of the stalk, and sugars in the middle and lower portion.

#### CROWNS AND ROOTS

Data from a few determinations on the juices of the crowns and roots of sorghum and corn are shown in Table 3. The crown samples consisted of the basal portion of the stalks below the surface of the soil with the roots and the cortical tissues removed. The root samples consisted of both large and small roots cut from the crown and wiped free from dirt with a dry cloth. In all the experiments the Kansas Orange sorgho and Sacaton June corn were grown in adjacent

<sup>5</sup> COLLIER, P. *SORGHUM; ITS CULTURE AND MANUFACTURE ECONOMICALLY CONSIDERED AS A SOURCE OF SUGAR, SYRUP, AND FODDER*. 570 p., illus. Cincinnati. 1884.

<sup>6</sup> HURD-KARRER, A. M. A CONCENTRATION GRADIENT IN CORNSTALKS. *Jour. Gen. Physiol.* 9: 341-343, 1926.

<sup>7</sup> COLLIER, P. *Op. cit.* (See footnote 5.)

rows. In 1928 and 1929 these two varieties were flanked by rows of Red kafir and Reid corn, from which samples were obtained. The Dwarf Yellow milo and Standard feterita mature rather early; Kansas Orange sorgho, Red kafir, and Sacaton June corn mature late but all three at about the same time.

The data in Table 3 indicate that the osmotic concentration of the juices of the crowns, as measured by the freezing-point depression, usually is higher in sorghum than in corn. The juice of the roots of sorghums has a much higher osmotic concentration than that of corn. Evidently corn roots do not carry nearly so much material in solution as do sorghum roots.

TABLE 3.—Freezing-point depression and specific conductivity on different dates of crown and root juices of four varieties of sorghum and two varieties of corn

Plant part and date of sampling	Freezing-point depression (° C.) of—						Specific conductivity (reciprocal ohms) of—					
	Dwarf Yellow milo	Red kafir	Standard feterita	Kansas Orange sorgho	Sacaton June corn	Reid corn	Dwarf Yellow milo	Red kafir	Standard feterita	Kansas Orange sorgho	Sacaton June corn	Reid corn
<b>Crown juices:</b>												
Aug. 15, 1927				1.15	0.96					0.0183	0.0288	
Aug. 27, 1927				1.82	1.51					.0273	.0451	
Aug. 24, 1928	1.21	0.92	1.18	1.05	.86	0.91	0.0127	0.0173	0.0157	.0121	.0234	0.0225
Aug. 30, 1928	1.01	1.01		1.05	.86	.92	.0213	.0272		.0183	.0373	.0301
Sept. 4, 1928 <sup>a</sup>		.89		.96	.94			.0190		.0128	.0267	
Aug. 22, 1929		1.08			1.06	1.13		.0187			.0292	.0316
Aug. 27, 1929		1.07			.89	.99		.0171			.0265	
<b>Root juices</b>												
Sept. 2, 1926				1.56	.76					.0178	.0215	
Aug. 10, 1927				1.28	1.06							
Aug. 27, 1927				1.83	1.49					.0266	.0349	
Aug. 30, 1928	1.12	1.11		1.21	.76	.83	.0251	.0206		.0238	.0309	.0311
Sept. 4, 1928 <sup>b</sup>		1.05		1.20	.83			.0174		.0182	.0230	
Aug. 22, 1929		1.28			.88	.90		.0201			.0252	.0240
Aug. 27, 1929		1.23			.80	.84		.0195			.0218	.0206

<sup>a</sup> Juice from less mature plants than samples taken Aug. 24 and 30, 1928.

<sup>b</sup> Juice from less mature plants than samples taken Aug. 30, 1928.

Conductivity determinations, on the other hand, show that the crowns and roots of corn contain considerably greater quantities of electrolytes than do sorghum crowns and roots. The higher freezing-point depression in sorghum crowns and roots is due, therefore, to nonelectrolytes, probably sugars. Conrad<sup>8</sup> has shown that considerable quantities of sugar are in sorghum roots. Wilson and Wilson<sup>9</sup> reported that sorghum roots contain nearly three times as much soluble organic matter as corn roots.

The higher osmotic concentration of the juices of crowns and roots may account in part for the fact that sorghum is better able than corn to resist drought. This high concentration would help to prevent tissue desiccation and might aid in removing water from a relatively dry soil.

The crowns and roots of sorghum plants often remain alive during extended periods of drought and send up tillers later, when moisture becomes available. Frequently a fair crop of grain sorghum is produced from the tillers alone.

<sup>8</sup> CONRAD, J. P. FERTILIZER AND LEGUME EXPERIMENTS FOLLOWING SORGHUMS. Jour. Amer. Soc. Agron. 20: 1211-1234, illus. 1928.

<sup>9</sup> WILSON, B. D., and WILSON, J. K. RELATION OF SORGHUM ROOTS TO CERTAIN BIOLOGICAL PROCESSES. Jour. Amer. Soc. Agron. 20: 747-754, 1928.

## TILLERS AND BRANCHES

Tillers develop from buds on the crown nodes of sorghum plants. Branches or offshoots, when present, develop on the sorghum stalk after the head is mature, from buds in the axils of the leaf sheaths. Table 4 shows the freezing-point depression and conductivity of the juices of leaves and internodes of main stalks, tillers, and branches on the same sorghum plants. Leaves from main stalks and from tillers of Dwarf Yellow milo and Dawn kafir show nearly the same freezing-point depression and conductivity, determined as the average of six plants. The internodes, on the other hand, show striking differences, the juice of main-stalk internodes being considerably greater in both freezing-point depression and conductivity than the juice from tiller internodes.

TABLE 4.—Freezing-point depression and conductivity of the leaf and internode juices and moisture content of main stalks, tillers, and branches of sorghum varieties

Variety and plant part	Freezing-point depression ( $^{\circ}$ C.) of—		Specific conductivity (reciprocal ohms) of—		Moisture content of internodes
	Leaves	Internodes	Leaves	Internodes	
Dwarf Yellow milo:					<i>Per cent</i>
Main stalks.....	1.01	1.37	0.0181	0.0149	-----
Tillers.....	1.03	.96	.0189	.0120	-----
Dawn kafir:					-----
Main stalks.....	.88	1.60	.0159	.0120	-----
Tillers.....	.81	1.13	.0157	.0091	-----
Standard feterita:					-----
Upper main stalks.....	.89	1.56	.0131	.0347	64.4
Lower main stalks.....	.87	1.61	.0169	.0257	79.1
Branches.....	.90	1.31	.0137	.0163	76.4
White durra:					-----
Upper main stalks.....	1.19	1.41	.0155	.0327	63.3
Lower main stalks.....	1.09	1.41	.0141	.0200	83.2
Branches.....	1.12	1.06	.0140	.0113	85.3
Black Amber sorgo:					-----
Upper main stalks.....	1.07	1.30	.0174	.0142	84.5
Lower main stalks.....	1.02	1.41	.0199	.0101	83.4
Branches.....	.89	1.13	.0113	.0063	85.9

Similar results were obtained by comparing the main stalks and branches of three sorghum varieties. Both tillers and branches are essentially young stalks that have not yet accumulated large quantities of stored materials. The leaf juices of the branches were very similar to those of the main stalks except in Black Amber sorgo. The internode juices of the branches, however, show considerably less freezing-point depression and conductivity than the juices from either the upper or lower internodes on the main stalks. The upper internodes consisted of the peduncle and second internode, both of which are above the base of the topmost branch.

Differences in moisture content of the branches and of the upper and lower main-stalk internodes, as shown in Table 4, may account in part for the differences in conductivity between the upper and lower main stalks in Standard feterita and White durra, but not in Black Amber sorgo.

## VARIETAL DIFFERENCES

Determinations of the freezing-point depression and conductivity of the juices of different sorghum varieties were made at various times during four seasons. Often the tests were limited to two or



three varieties sampled on a single day. No two varieties were at exactly the same stage of maturity at a given time, and all varieties were changing rapidly during growth and maturation. Differences in soil and soil moisture and in the condition of the plants may have affected the results considerably. Table 5 shows the measurements of average and extreme freezing-point depression and conductivity of the juices of seven varieties of sorghum and one variety of corn. The data include measurements of composite samples of the juice of all or most of the leaves or internodes of a plant and averages of the determinations of individual leaves or internodes.

The data in Table 5 indicate a wide variability in the character of plant juices sampled under different conditions of season, maturity, and moisture. The average results for each variety indicate that in both freezing-point depression and conductivity only small differences exist between the leaf juices of different varieties.

The leaf juices of Sacaton June corn were higher than those of any sorghum in both characters. Larger varietal differences occurred in the stalks than in the leaves, the two sorghos and Standard feterita showing the greatest freezing-point depression and Sacaton June corn the least. In conductivity of stalk juices, Standard feterita was the highest, followed by Dwarf Yellow milo. The two sorghos showed the lowest conductivity. Of the sorghum varieties, feterita showed the highest conductivity of internode juices and the lowest conductivity of leaf juices, whereas Kansas Orange sorgho showed the lowest conductivity in the stalks and the highest in the leaves. These relationships did not hold for all varieties.

TABLE 5.—Maximum, minimum, and average freezing-point depression and conductivity of the leaf and internode juices of seven varieties of sorghum and one variety of corn at Sacaton, Ariz., during four seasons

#### FREEZING-POINT DEPRESSION

Variety	Leaves				Internodes			
	Samp- les	Maxi- mum	Mini- mum	Aver- age	Samp- les	Maxi- mum	Mini- mum	Aver- age
	Num- ber	°C.	°C.	°C.	Num- ber	°C.	°C.	°C.
Dwarf Yellow milo.....	23	1.22	0.85	1.00	20	1.55	0.91	1.25
Dawn kafir.....	21	1.16	.79	.91	18	1.77	.97	1.41
Reed kafir.....	17	1.21	.79	.95	13	1.65	.98	1.28
Dwarf hegari.....	18	1.23	.78	1.01	14	1.92	.85	1.37
Standard feterita.....	17	1.12	.77	.90	14	1.76	1.23	1.50
Black Amber sorgho.....	17	1.17	.66	.92	13	1.58	1.23	1.35
Kansas Orange sorgho.....	19	1.35	.86	1.11	15	2.10	1.01	1.61
Sacaton June corn.....	20	1.81	.89	1.16	15	1.76	.79	1.19

#### SPECIFIC CONDUCTIVITY

	Num- ber	Recipro- cal ohms	Recipro- cal ohms	Recipro- cal ohms	Num- ber	Recipro- cal ohms	Recipro- cal ohms	Recipro- cal ohms
Dwarf Yellow milo.....	23	0.0206	0.0133	0.0169	20	0.0327	0.0116	0.0193
Dawn kafir.....	21	.0234	.0140	.0167	18	.0192	.0100	.0129
Reed kafir.....	17	.0201	.0152	.0176	13	.0247	.0123	.0170
Dwarf hegari.....	18	.0229	.0144	.0168	14	.0219	.0091	.0129
Standard feterita.....	17	.0209	.0121	.0150	14	.0321	.0141	.0222
Black Amber sorgho.....	18	.0180	.0113	.0154	14	.0156	.0072	.0111
Kansas Orange sorgho.....	19	.0212	.0145	.0178	15	.0117	.0060	.0084
Sacaton June corn.....	20	.0330	.0150	.0215	14	.0216	.0063	.0143

TABLE 6.--Freezing-point depression and conductivity of tissue fluids, and moisture content of internodes of 16 varieties of sorghum and 2 varieties of corn at Sacaton, Ariz., 1928 and 1929

## FREEZING-POINT DEPRESSION (°C.)

Group and variety	Leaf juices sampled						Internode juices sampled						Average moisture content of internodes <sup>a</sup>
	1928			1929			1928			1929			
	Aug. 24 or 25	Aug. 31 or Sept. 3	Aug. 20 or 21	Aug. 24 or 25	Aug. 30 or 31	Average	Aug. 24 or 25	Aug. 31 or Sept. 3	Aug. 20 or 21	Aug. 24 or 25	Aug. 30 or 31	Average	
Milo:													
Dwarf Yellow . . . . .	1.16	1.02	0.90	1.03	0.86	0.99	1.30	1.52	1.27	1.41	1.21	1.34	
Peterita:													
Standard . . . . .	1.01	.92	.78	.83	.77	.86	1.45	1.47	1.44	1.53	1.38	1.45	
Durra:													
White . . . . .	1.04		.95	.90	.99		1.10		1.65	1.53	1.60		
Kafir:													
Dawn . . . . .	1.16	.86	.80	.89	.79	.90	1.77	1.68	1.48	1.71	1.20	1.57	
Reed . . . . .	1.21	.97	.79	.92	.82	.94	1.27	1.46	1.25	1.34	1.16	1.30	
Sunrise . . . . .	1.34	.97	.95	.97	.92	1.03	1.26	1.57	1.40	1.58	1.42	1.45	
Texas Blackhull . . . . .	1.12	.90					1.24	1.42					
Red . . . . .	1.15	1.12	.95	.91	.93	1.01	1.24	1.30	1.34	1.31	1.49	1.34	
Hegari:													
Dwarf . . . . .	.97	.91	.85	1.04	.93	.94	1.30	1.21	1.13	1.27	1.64	1.31	
Kaoliang:													
Manchu Brown . . . . .			1.00	.86	1.01				1.53	1.44	1.61		
Sorgo:													
Black Amber . . . . .	1.17	.97	.89	.93	.86	.96	1.44	1.42	1.24	1.11	1.21	1.28	
Kansas Orange . . . . .	1.15	1.12	.93	1.05	.89	1.03	1.43	1.80	1.40	1.58	1.64	1.57	
Leoti Red . . . . .	1.30	1.00	1.07	.94	.98	1.06	1.43	1.54	1.52	1.58	1.51	1.52	
Fred . . . . .	1.09	.99	.92				1.37	1.42	1.37				
Atlas . . . . .			1.02	.92	.98				1.48	1.63	1.50		
Broomcorn:													
White Italian . . . . .			1.28	1.01	1.10				1.50	1.56	1.47		
Corn:													
Sueaton June . . . . .	1.29	1.08	.92	1.13	1.16	1.12	1.17	.99	1.07	1.02	1.13	1.08	
Reid . . . . .	1.51	1.00	1.10	1.20	1.02	1.17	1.26	.89	1.04	1.25	1.36	1.16	

## CONDUCTIVITY (RECIPROCAL OHMS)

Milo:															
Dwarf Yellow . . . . .	0.0178	0.0172	0.0167	0.0162	0.0163	0.0168	0.0131	0.0142	0.0128	0.0144	0.0162	0.0141	82.9		
Peterita:															
Standard . . . . .	.0140	.0153	.0137	.0160	.0162	.0150	.0141	.0204	.0190	.0213	.0238	.0197	81.2		
Durra:															
White . . . . .	.0157		.0158	.0155	.0155		.0161		.0203	.0208	.0216		77.3		
Kafir:															
Dawn . . . . .	.0169	.0184	.0161	.0163	.0171	.0170	.0101	.0126	.0103	.0103	.0156	.0119	82.0		
Reed . . . . .	.0189	.0188	.0168	.0179	.0188	.0182	.0123	.0146	.0138	.0136	.0180	.0145	84.0		
Sunrise . . . . .	.0161	.0184	.0174	.0173	.0174	.0173	.0101	.0117	.0158	.0168	.0161	.0141	82.3		
Texas Blackhull . . . . .	.0163	.0175					.0157	.0189					81.8		
Red . . . . .	.0170	.0181	.0170	.0162	.0158	.0168	.0129	.0169	.0144	.0168	.0161	.0164	84.7		
Hegari:															
Dwarf . . . . .	.0155	.0229	.0151	.0154	.0172	.0172	.0116	.0122	.0104	.0120	.0142	.0121	83.3		
Kaoliang:															
Manchu Brown . . . . .			.0197	.0170	.0179				.0229	.0261	.0277		77.1		
Sorgo:															
Black Amber . . . . .	.0142	.0180	.0165	.0176	.0177	.0168	.0077	.0116	.0098	.0131	.0143	.0113	85.6		
Kansas Orange . . . . .	.0153	.0143	.0157	.0177	.0175	.0161	.0069	.0074	.0085	.0112	.0082	.0084	86.2		
Leoti Red . . . . .	.0171	.0165	.0187	.0180	.0171	.0175	.0088	.0118	.0122	.0123	.0126	.0115	82.8		
Fred . . . . .	.0175	.0173	.0173				.0124	.0176	.0151				82.3		
Atlas . . . . .			.0174	.0163	.0162				.0133	.0142	.0147		85.2		
Broomcorn:															
White Italian . . . . .			.0223	.0177	.0187				.0266	.0272	.0291		78.5		
Corn:															
Sueaton June . . . . .	.0199	.0225	.0168	.0179	.0203	.0196	.0122	.0122	.0116	.0201	.0216	.0155	81.7		
Reid . . . . .	.0221	.0236	.0197	.0196	.0190	.0203	.0096	.0090	.0180	.0181	.0122	.0134	83.6		

\* Average of 4 determinations, 2 each year.

Determinations made on 16 sorghum and 2 corn varieties in 1928 and 1929 are shown in Table 6. These samples were obtained under average and fairly comparable conditions. The most obvious distinction between the juices of sorghum varieties is in the conductivity of the juices of the internodes. In general there seems to be a tendency for varieties within the different groups of sorghums to show similar conductivities. Feterita, durra, broomcorn, and kaoliang, which have dry or pithy stalks, have a high conductivity of the internode juices. Collier<sup>10</sup> showed durra stalk juices to be considerably higher than sorgos in "solids" (mostly salts and organic acids). The sorgos have a comparatively low conductivity of stalk juices.

The kafirs and the milo seem to be intermediate in conductivity and in stalk juiciness between the very dry-stalked sorghums and the sorgos. Dwarf hegari is similar to kafir in the juiciness of the stalk, in the appearance of the heads and seeds, and in tissue-fluid properties.

The leaf juices of broomcorn and corn have a greater freezing-point depression and conductivity than was obtained in the grain sorghums and sorgos. All sorghums exceed corn in the freezing-point depression of the stalk juices.

#### RELATION TO MOISTURE CONTENT

The stalks of feterita, durra, broomcorn, and kaoliang are rather dry, whereas kafir stalks are juicy and sorgo stalks extremely juicy. This indicates a general tendency for juiciness to be inversely proportional to the conductivity of the juice. Some moisture-content determinations were made in 1928 and 1929 to test this relationship. Portions of the peeled internodes prepared for the juice extraction were saved for the moisture determinations, and the average moisture content of these is shown in Table 6. The moisture content of the internodes of 14 sorghum varieties in 1928 ranged from 78 to 87 per cent, and in 1929 the average moisture content of duplicate collections of 13 varieties varied from 77 to 89 per cent.

There was some relationship between the moisture content of the stalks and the conductivity of the tissue fluids in sorghum stalks, a negative but not significant correlation coefficient of  $-0.28 \pm 0.12$  being obtained in 1928 and a larger and quite significant coefficient of  $-0.61 \pm 0.08$  in 1929.

There was no definite relation between the moisture content of stalks and the freezing-point depression of the juices, because sorgo stalks that are very juicy also contain considerable quantities of dissolved sugar.

#### SEASONAL CHANGES

No determinations were made on young sorghum plants, because of the difficulty of getting samples of comparable leaves and internodes before the plants reach the heading stage. Changes that took place between heading and ripening, however, are shown by the data for the three periodic samplings of 1926 (Tables 1 and 2) and by the differences between main stalks and tillers (Table 4). There was little change in either freezing-point depression or conductivity of the leaf juices as the plants approached maturity. In the internodes, how-

<sup>10</sup> COLLIER, P. Op. cit. (See footnote 5.)

ever, there was a marked accumulation of soluble materials in the juices as the stalks neared maturity. Both the freezing-point depression and the conductivity may increase 50 to 75 per cent within a month. Collier<sup>11</sup> showed average changes between heading and ripening as follows: Sucrose increased from 4.23 to 11.75 per cent of the juice, glucose decreased from 3.89 to 2.09 per cent, "solids not sugars" increased from 2.36 to 2.82 per cent, while the specific gravity increased from 1.043 at the heading stage to 1.068 at maturity.

## EFFECT OF MOISTURE SUPPLY

In order to determine the changes that occur when the plants are subjected to the stress of drought, a plot on which seven varieties of sorghum and one of corn were growing was left unirrigated during a dry period in 1927. Another plot, similarly prepared, was kept supplied with irrigation water as needed. After the leaves on the plants in the delayed-irrigation plot had begun to show severe firing, samples were taken and the land was irrigated the following day. On the day following this irrigation, samples again were taken. Table 7 shows the data obtained before and after the delayed irrigation, together with comparable data from samples from the plot receiving normal irrigation.

TABLE 7.—Freezing-point depression and specific conductivity of the leaf and internode juices of seven varieties of sorghum and one variety of corn from a dry plot before and after a delayed irrigation and from a plot normally irrigated

Variety	Freezing-point depression (° C.) under indicated treatment						Specific conductivity (reciprocal ohms) under indicated treatment					
	Leaf juices			Internode juices			Leaf juices			Internode juices		
	Delayed irrigation		Normal irrigation	Delayed irrigation		Normal irrigation	Delayed irrigation		Normal irrigation	Delayed irrigation		Normal irrigation
	Before	After		Before	After		Before	After		Before	After	
Dwarf Yellow milo.....	0.89	0.92	1.06	1.03	1.30	1.19	0.0159	0.0175	0.0189	0.0275	0.0281	0.0286
Dawn kufir.....	.96	.92	1.03	1.45	1.31	1.39	.0195	.0184	.0168	.0120	.0153	.0132
Reed kufir.....	1.06	.96	.92	1.07	1.17	.98	.0183	.0201	.0168	.0247	.0198	.0220
Dwarf hegari.....	.95	1.20	1.23	.85	1.02	1.54	.0175	.0204	.0156	.0129	.0116	.0157
Standard foterita.....	.....	.....	1.03	1.70	.....	1.76	.0121	.....	.0209	.0214	.....	.0308
Black Amber sorgho.....	1.07	.....	.98	1.39	.....	1.42	.0171	.....	.0129	.0137	.....	.0156
Kansas Orange sorgho.....	1.27	1.29	1.35	2.10	1.91	1.99	.0174	.0212	.0184	.0086	.0091	.0071
Average of 7 varieties.....	1.02	.....	1.09	1.37	.....	1.47	.0168	.....	.0172	.0173	.....	.0190
Average of 5 varieties.....	1.02	1.06	1.13	1.30	1.52	1.42	.0177	.0195	.0173	.0174	.0168	.0173
Sacaton June corn.....	1.81	1.46	1.22	1.76	1.41	1.21	.0330	.0250	.0234	.0161	.0146	.0132

The average results for the seven sorghum varieties show that the freezing-point depression and conductivity of the juices of both leaves and internodes were slightly greater in the plants receiving normal irrigation than in plants from which water was withheld. This shows that the osmotic concentration of sorghum juices is not increased by water deficiency. The juices of the leaves and internodes of Sacaton June corn showed a higher concentration in all cases in the plants

<sup>11</sup> COLLIER, P. Op. cit. (See footnote 5.)

from which water was withheld than in the normally irrigated plants. The corn plants were severely wilted, but the sorghum plants showed only slight wilting; this probably explains the differences found in the tissue fluids of the two species.

Only five varieties of sorghums were sampled after the delayed irrigation, because the Standard feterita and Black Amber sorgo were mature at this time. Increases in the average freezing-point depression of both leaves and internodes of the sorghums and in the conductivity of the leaves were obtained after the delayed irrigation. This apparently indicated a resumption of nutrient absorption and of photosynthesis by the sorghums. The corn-plant juices, on the other hand, all showed definite decreases in freezing-point depression and conductivity after the delayed irrigation, indicating that the absorption of water was the most important change taking place.

Collier<sup>12</sup> found a difference of only 0.003 in the average specific gravity of the juice of 36 varieties of sorgo taken before and after a heavy rainfall following a long drought.

#### TOTAL SOLIDS AND BOUND WATER

The percentage of total solids in the juices, as measured by the sugar scale of the Abbe refractometer, was determined in a considerable number of samples of leaf and internode juices in 1926. The total-solids measurements corresponded in general with the freezing-point depression determinations but were determined less accurately. The total solids in the leaf juices of sorghums varied from 6.5 to 14.3 per cent and those in Sacaton June corn from 6.2 to 13.7 per cent. The total solids of the internode juices varied from 7.2 to 19.4 per cent in sorghums and from 10.1 to 11.9 per cent in Sacaton June corn.

The method used showed no indication of bound water in the leaf juices that could not be accounted for by experimental error. Internode juices apparently contained no bound water before the plants were nearly mature. When nearing maturity, however, there was some indication of bound water in the internode juices of sorghum but none in corn. Irregularities in the data preclude definite conclusions, but the presence of bound water, even in the stalks, appears doubtful.

#### CONCLUSIONS AND SUMMARY

The freezing-point depression is greatest in the juices of the terminal leaf of sorghums, with a progressive decrease in the leaf juices from the top of the plant downward.

The freezing-point depression of the juices of different internodes is about the same in the flowering stage but increases from the top down to the fourth internode when the plant approaches maturity.

The conductivity of the juices of leaves in different positions is nearly the same.

The juices of the upper internodes have the lowest conductivity in the flowering stage and the highest in the milk or dough stage.

The crowns and roots of sorghums have a higher osmotic concentration than those of corn, but a lower conductivity. The difference in osmotic concentration may partly account for the greater drought resistance of sorghums.

<sup>12</sup> COLLIER, P. Op. cit. (See footnote 5.)

The juices of tillers and branches of sorghums have less freezing-point depression and a lower conductivity than the older main stalks.

The leaf juices of sorghums show only small varietal differences. The conductivities of the internode juices show considerable varietal differences and are high in feterita and durra, low in sorgos, and intermediate in kafirs.

Sorghum-leaf juices (except broomcorn) have less freezing-point depression and a lower conductivity than corn.

Sorghum-stalk juices usually have a greater freezing-point depression than those of corn. The average conductivity of sorghum-stalk juices is about the same as that of corn.

The differences in conductivity of stalk juices in sorghums can be accounted for to a considerable extent by differences in the juiciness of the stalks.

There is little change in either freezing-point depression or conductivity of leaf juices as the plants approach maturity. The internode juices may increase 50 to 75 per cent in freezing-point depression and specific conductivity between heading and ripening.

Water deficiency does not cause an increase in the freezing-point depression or conductivity of the leaf and stalk juices in sorghum, but may in corn if the plants wilt.

There was no definite indication of bound water in the juices of sorghums.



# GERMINATION LOSS OF CONIFEROUS SEEDS DUE TO PARASITES<sup>1</sup>

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## INTRODUCTION

Literature on damping off (2, 3, 8, 18, 22, 30, 34)<sup>2</sup> contains scattered references to germination loss caused by damping-off fungi to various hosts, but there are comparatively few references to that of conifers. Spaulding (32, p. 74), in discussing the damping off of coniferous seedlings, reported that "while damping-off may kill the germinating seedlings before they push through the soil, it usually attacks them a few days after the seedlings have come up." Hartley (10, 11, 12) stated that *Pythium debaryanum*, *Corticium ramorum* (*Phizoc-tonia solani*), and *Fusarium* spp. all cause damping-off of coniferous-seed beds. The best data on damping-off under nursery conditions are furnished by Hart (13). He found that the methods of the results with several species of fungi and disinfection tests. He found evidence that damping-off always occurred, that in some cases the damping-off seedlings emerged from the soil was apparently considerably greater than the damping off that took place later, and that in extreme cases the estimated germination loss equaled approximately four-fifths of the total number of viable seeds. These disinfectant tests gave little indication as to which fungi were responsible for the loss. Inoculation experiments in which inoculum had been added to the soil instead of being placed in direct contact with the seeds or seedlings also did not show whether certain fungi failed to cause germination loss because of lack of parasitic ability or because of inability to grow well in the soils used. None of the previous tests furnished data as to whether the fungi not only caused a very early rotting of the radicles but also decayed viable unruptured seeds. This information is important in determining whether seeds planted in the fall and those that normally do not germinate until the second or third year after planting will be attacked by damping-off fungi while dormant. In order to secure this information coniferous seeds were directly inoculated with damping-off fungi. Results secured by the direct inoculation of stems, taproots, and older roots with the same fungi can be found elsewhere (27, 28, 29).

## METHODS OF INOCULATION

A few small preliminary trials (26) in which seeds were sown broadcast on the surface of agar of Petri-dish cultures of the tested fungi demonstrated this method to be very unsatisfactory. Thereupon the agar was removed from two rectangles 2½ inches long and five-eighths inch wide in each Petri-dish fungus culture. The seeds were then arranged on the glass just inside the edges of the remaining agar adjacent to the rectangular space, with one side of each seed in contact with the agar. The fungi were grown in all cases on corn-meal

<sup>1</sup> Received for publication Sept. 24, 1930; issued January, 1931.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 91.



agar. In each experiment one plate was inoculated with each fungus line. Throughout this paper a fungus culture arising from one isolation is called a line.

Seeds arranged along the perimeters of similar rectangles cut in sterile corn-meal agar served as controls. This sterile agar was part of the same batch of medium on which the inoculating fungi were grown. The uninoculated or control plates were made under the same conditions as the inoculated ones. They usually became overgrown with *Mucor* or *Rhizopus* because the seeds were not sterilized before inoculation, but this was not considered a disadvantage, because it made the moisture conditions in the uninoculated plates more nearly like those in the inoculated ones. The facts that the pine radicles in all uninoculated plates were entirely free from rot and that in spruce there was only an insignificant amount of radicle decay in one uninoculated plate suggest that this fungus was not much of a parasite under the conditions of these experiments. In each of the experiments numbered 100, 116, and 134, there were 25 uninoculated plates, and the probable error of the mean germination of these 25 has been calculated. The distribution of the figures from these uninoculated plates was approximately symmetrical. For each of the other experiments only five or six uninoculated plates were used.

Each plate in an experiment received the same quantity of seeds, which was determined by measure rather than by count. This, of course, introduced a certain small amount of error, probably partly compensated by the fact that very small seeds have a low germination percentage. In securing reliable results, the emphasis was placed on testing numerous lines of each important fungus and several replications for each line, rather than on extreme precision in the individual tests. The seed measure used for each plate in the experiments with *Pinus banksiana* Lamb. was large enough to hold on the average 100 seeds, in those with *P. resinosa* Ait. 75 seeds, and in those with *Picea engelmanni* Engelm. 200 seeds. After being seeded the plates were placed in a dark cupboard in the laboratory.

The experiments were continued until germination was practically complete, usually about 10 days after sprouting began and approximately 3 weeks after the seeds were sown on the plates. Results from plates that became dry before the ends of the experiments were discarded.

The method described exposed to infection not only the unruptured seeds but also radicles as they emerged from the seed coats. When the radicles were not attacked by the fungi, the seedlings developed fairly normal hypocotyls and cotyledons in the Petri dishes.

A second method (26) of inoculating radicles was to germinate the seeds on filter paper and to place the radicles of the seedlings in contact with rectangles of inoculated corn-meal agar in sterile Petri dishes. In this case, too, sterile agar was used for the controls. Ten seedlings served as a unit.

The principal fungi<sup>3</sup> tested by these methods were *Pythium ultimum*,<sup>4</sup> *Corticium vagum* B. and C. (*Rhizoctonia solani* Kühn), *Fusarium* spp., and *Botrytis* spp.

<sup>3</sup> In the experiments described in this paper the writer used Carl Hartley's cultures. The source and host of each of these cultures are given in an earlier paper (26, Table I). The writer wishes to thank Doctor Hartley for his kind suggestions, assistance, and criticisms regarding this investigation and this paper, especially the statistical portions.

<sup>4</sup> Formerly called *Pythium debaryanum* Hesse, but now *P. ultimum* seems the more probable name. However, no zoospores have been secured.

## RESULTS OF THE PRESENT INVESTIGATION

The average number of seeds that germinated in units inoculated with the various fungi is shown in Table 1.

TABLE 1.—Average number of seeds germinating in the various units  
[Fungi arranged in alphabetical order]

Fungi tested	Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131
	Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120		
Botrytis spp. (small-sclerotium type).....	67	—	60	—	50	—
Botrytis cinerea.....	47	—	54	59	45	38
Cephalothecium roseum.....	36	—	—	—	—	—
Corticium vagum (more virulent lines) <sup>a</sup> .....	46	52	56	—	50	16
Corticium vagum (less virulent lines) <sup>a</sup> .....	55	46	62	55	50	54
Fusarium spp. (exclusive of No. 906).....	54	45	57	53	45	32
Fusarium acuminatum.....	59	—	54	56	42	—
Fusarium culmorum group.....	56	—	57	—	38	—
Fusarium discolor section.....	58	—	58	53	60	—
Fusarium elegans group.....	54	54	57	51	46	—
Fusarium moniliforme species and section.....	49	46	56	57	42	—
Fusarium solani.....	74	—	61	51	44	32
Fusarium sporotrichioides.....	41	33	37	—	35	17
Fusarium vasinfectum.....	57	—	65	—	—	—
Gibberella saubinetii.....	58	42	63	41	48	—
Mucor racemosus.....	—	—	63	59	—	—
Pestalozzia funerea (?).....	43	—	53	50	—	—
Phomopsis juniperovora.....	50	—	50	—	26	—
Phycomycete (unidentified).....	61	—	—	—	—	—
Phytophthora sp.....	48	—	60	36	45	—
Phytophthora cactorum.....	61	—	44	—	—	—
Phthiactydis citrophthora.....	42	—	56	—	—	—
Pythium aphanidermatum.....	37	—	41	—	41	—
Pythium artotrogus.....	58	—	61	—	43	—
Pythium ultimum.....	46	—	50	48	45	39
Rhizoctonia potomacensis <sup>b</sup> .....	50	—	—	—	—	—
Thielavia basicola.....	47	—	66	—	—	—
Verticillium sp.....	—	—	52	—	—	—
Uninoculated.....	53	55	58	42	53	57

<sup>a</sup> Classified in accordance with apparent virulence to radicles.

<sup>b</sup> Probably a line of *Corticium vagum* according to H. A. Edson, who furnished it.

## RADICLE DECAY

<sup>1</sup> In Tables 2 to 8 are given the percentages of radicles of *Pinus banksiana*, *P. resinosa*, and *Picea engelmanni* that were decayed by each fungus line when tested by the first method described. Each percentage figure was weighted by the number of inoculated radicles, which in each plate equaled the number of seeds that germinated. In these tables the lines are arranged in decreasing order of apparent virulence, due consideration being given to a line and its reisolations. The average number of inoculated radicles in each experiment is given in Table 1 and the average radicle decay in each experiment in Table 8.

## PYTHIUM ULTIMUM

In every experiment all the tested lines of *Pythium ultimum* were able to cause decay of young radicles. (Tables 2 and 8.) Most of them, including No. 767, which was only slightly virulent according to Hartley's (11) data, would probably be able to cause heavy germination loss in seed beds by killing young radicles soon after they emerge from the seed coats. There was no significant difference between the average radicle decay caused by lines isolated from coniferous hosts and by those isolated from nonconiferous hosts. (Table 8.) The

data were insufficient to prove whether there were real differences in host susceptibility, but there were some indications that the spruce radicles were more resistant than those of pine to certain lines.

The few experiments conducted by the second method failed to confirm the apparently greater resistance of spruce radicles to certain lines of *Pythium ultimum*. Although these results were based on inoculated units of only 10 seedlings, all the uninoculated radicles remained healthy, while the majority of the inoculated ones decayed. Two lines of *P. ultimum* killed 65 per cent of the 20 inoculated radicles of *Pinus banksiana*; six lines killed 97 per cent of the 60 of *P. resinosa*, and nine lines 89 per cent of the 110 of *Picea engelmanni*.

Other writers have found that *Pythium* can also cause germination loss to such unrelated hosts as sugar beets (3, 25), *Robinia pseudacacia* (14), and tobacco (20).

TABLE 2.—Percentages of coniferous radicles decayed by various lines of *Pythium ultimum*

Line No.	Pinus banksiana			Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average
	Experiment No. 100	Experiment No. 116	Experiment No. 120			
810 <sup>a</sup> .....	100	100	-----	100	100	100
550.....	100	98	-----	100	100	100
258.....	81	93	-----	100	86	91
131 <sup>a</sup> .....	-----	95	100	100	94	97
408 <sup>ab</sup> .....	100	92	-----	-----	-----	90
338 <sup>ab</sup> .....	-----	100	96	100	12	85
295 <sup>ab</sup> .....	100	100	-----	89	20	81
767.....	100	100	-----	90	27	83
296 <sup>a</sup> .....	82	87	-----	100	14	74
529 <sup>a</sup> .....	58	35	-----	36	-----	42
743.....	2	23	-----	-----	93	40
Average uninoculated unit.....	0	0	0	0	.4	.1

<sup>a</sup> Lines isolated from nonconiferous hosts; no oospores have been observed for line 810.

<sup>b</sup> Reisolation of line No. 131.

#### OTHER PHYCOMYCETES

Two additional Phycomycetes (Tables 3 and 8), *Pythium aphanidermatum* Edson (Fitz)<sup>5</sup> and *Pythiacystis citrophthora* Sm. and Sm., showed as much apparent ability as *Pythium ultimum* to decay young radicles. However, despite the large amount of diagnostic work reported by Hartley (11), neither of them has been found occurring naturally in coniferous seed beds. *Phytophthora* sp. and *P. cactorum* (C. and L.) Sebrath appeared slightly parasitic to young radicles, whereas the other tested Phycomycetes gave no evidence of parasitism. According to Hartig (8), *P. fagi* kills seedlings of beech before they reach the surface of the soil.

<sup>5</sup> Called *Rheosporangium aphanidermatus* in a previous paper (29, Table I).

TABLE 3.—Percentages of coniferous radicles decayed by various *Phycomycetes*

Fungus	Line No.	Pinus banksiana			Pinus resinosa, experiment No. 134	Average
		Experiment No. 100	Experiment No. 116	Experiment No. 120		
<i>Pythium aphanidermatum</i> .....	{ a 351	100	97	-----	100	99
	a b 430	51	100	-----	-----	77
<i>Pythiacystis citrophthora</i> .....	{ a 943	81	84	-----	-----	83
	c 843	0	58	-----	-----	32
<i>Phytophthora</i> sp.....	{ 358	7	4	-----	26	11
	372	0	-----	8	4	4
<i>Phytophthora cactorum</i> .....	{ a 901	0	32	-----	-----	13
	778	0	-----	-----	-----	0
<i>Pythium artotrogus</i> .....	{ 821	0	0	-----	0	0
	833	0	-----	-----	-----	0
Unidentified <i>Phycomycete</i> .....	{ a 543	0	-----	-----	-----	0
<i>Mucor racemosus</i> .....	a 927	0	0	0	-----	0
Average uninoculated unit.....	-----	0	0	0	0	0

a Lines isolated from nonconiferous hosts.

b Reisolation of line No. 351.

c Reisolation of line No. 372.

## CORTICIUM VAGUM

Only the most virulent third (Tables 4 and 8) of the lines of *Corticium vagum* approximated the ability of *Pythium ultimum* to decay young radicles. Several of the *C. vagum* lines behaved as pure saprophytes, decaying none of the inoculated radicles. In many units sclerotia of *C. vagum* were produced on the exterior of the seeds. Even the more virulent *C. vagum* lines seemed less destructive than they did in Hartley's experiments (11). It is possible that the apparently greater importance of *C. vagum* in causing germination loss in nursery beds and in soil experiments is due to its ability to spread through soil more readily than *P. ultimum* does. A more important conclusion is that among the tested fungi these two are apparently the only important ones causing decay of radicles under natural conditions (12). In view of the fact that some lines of *C. vagum* are known to cause disease only at low temperatures, it is possible that experiments conducted at temperatures lower than the ordinary laboratory ones might result in the production of more radicle decay by this fungus. Balls (1) found that the "sore-shin" fungus vigorously attacked germinating cottonseeds at 20° C., but that it caused only slight superficial lesions at 33° and no injury at 37°.

On the whole, *Corticium vagum* decayed fewer radicles by the second than by the first method of inoculation. Six of the more virulent lines killed 73 per cent of the 60 inoculated radicles of *Pinus resinosa*, and seven killed 36 per cent of the 70 inoculated radicles of *Picea engelmannii*. Three and five of the less virulent lines killed none of the inoculated radicles of *Pinus banksiana* and *P. resinosa*, respectively, while nine of them killed 17 per cent of the 110 inoculated radicles of *Picea engelmannii*.

Rhizoctonia seems also to vary in its virulence to other hosts, as it is known to cause germination loss in tobacco seed beds (20) and to kill germinating seeds of cotton (?), but it did not prevent bean seeds from germinating (13).

TABLE 4.—Percentages of coniferous radicles decayed by various lines of *Corticium vagum*

Line No.	Pinus banksiana				Pinus resinosa, experiment No 134	Picea engelmanni, experiment No. 131	Average
	Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120			
More virulent lines:							
183 <sup>a</sup> .....	100		98		80		94
187 <sup>a</sup> .....	69		68		100		79
213 <sup>a</sup> .....	99		65		100	25	83
331 <sup>a b</sup> .....	100	76	13		100		74
340 <sup>a b</sup> .....	82	45	75		92		71
332 <sup>c</sup> .....	100		91		100		98
333 <sup>c</sup> .....	52		95		96		85
329 <sup>c</sup> .....		54	94		100		75
147 <sup>d</sup> .....	41	8	40				33
343 <sup>c d</sup> .....			2				2
746 <sup>e</sup> .....	100		82				88
747 <sup>d e</sup> .....	92		29		7		37
Less virulent lines:							
240.....			50		13		35
205.....	0		66		0		24
50.....			23	14			19
341 <sup>a f</sup> .....	32	26	78		20	4	32
233 <sup>a f</sup> .....	8	10	50		5	30	23
230 <sup>a</sup> .....		2	1		2		2
330 <sup>a f</sup> .....	0		2		0	0	1
761.....	11	0		16	19		11
724.....	0		2		32		10
340.....	10		1				5
361.....	8		1		4		4
362.....	2			0			1
723.....				0			0
721.....			0				0
352.....			0				0
365.....	0		0				0
363.....	0	0	0				0
189 <sup>a</sup> .....	0		0				0
186 <sup>a</sup> .....	0		0				0
Average uninoculated unit.....	0	0	0	0	0	.4	1

<sup>a</sup> Lines isolated from nonconiferous hosts.<sup>b</sup> Reisolation of line No. 213.<sup>c</sup> Reisolation of line No. 147.<sup>d</sup> Included because of relation to other lines in the more virulent group.<sup>e</sup> Duplicate of line No. 746.<sup>f</sup> Reisolation or duplicate of line No. 230.

## FUSARIUM SPP.

*Fusarium sporotrichioides*, which has never yet been isolated from damped-off coniferous seedlings (11), was the only species of *Fusarium* (Tables 5 and 8) that approached *Pythium ultimum* in ability to decay radicles. One other *Fusarium* culture, which was omitted from the table because it was later found to contain two species (*F. moniliforme* and *F. discolor* <sup>6</sup>), also showed decided ability to attack radicles.

Some lines of the *moniliforme* group of *Fusarium* also gave some evidence of ability to decay radicles. Many of these lines were 7 years old when they were used in these experiments and had lost their pigment-forming capacity. This fungus is also known to cause root rot of corn (2, 34) and under certain conditions to inhibit germination (22). *Fusarium discolor sulphureum* and *F. arthrosporioides* also showed consistent ability to attack radicles.

The other *Fusarium* species, even those that were fairly parasitic to older seedlings (29), either showed little ability to attack radicles or behaved as pure saprophytes. *Fusarium acuminatum*, *F. ventricosum*, *F. orthoceras*?, *F. avenaceum*, *F. hyperoxysporum*, *F. coeruleum*,

<sup>6</sup> Determined by Helen Johann.

*F. oryzae*, *F. radicola*, 3 lines of the *moniliforme* section, 3 lines of the *discolor* section, 1 line of *Gibberella saubinetii*, 5 lines of the *F. elegans* group, 2 lines of the *F. culmorum* group, 2 lines of the *F. solani* group, and 2 unidentified species of *Fusarium* showed consistent lack of ability to attack radicles. The remaining lines were sometimes parasitic and at other times nonparasitic to young radicles. These results confirm the earlier conclusions based on soil inoculation tests with *F. moniliforme* and *F. ventricosum* (12).

TABLE 5.—Percentages of coniferous radicles decayed by various lines of *Fusarium*, *Gibberella*, and *Nectria*

Fungus	Line No.	Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average
		Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120			
<i>Fusarium sporotrichioides</i> .....	a 906	100	54	84	—	86	100	84
<i>Fusarium discolor sulphureum</i> .....	a 936	19	—	16	—	—	—	17
	a 948	59	—	4	—	86	—	30
	247	53	—	0	—	—	—	28
	266	6	—	—	8	17	—	10
	a 949	0	—	0	—	38	—	10
	a 308	0	—	2	—	4	—	2
	a 910	7	—	0	—	0	—	2
<i>Fusarium moniliforme</i> species and section.	273	0	2	2	—	—	—	1
	a 947	0	—	0	—	4	—	1
	239	—	—	3	0	0	—	1
	249	0	5	—	0	0	—	1
	251	—	—	0	0	0	—	0
	260	0	—	0	—	0	—	0
	265	0	—	0	—	0	—	0
<i>Fusarium arthrosporioides</i> .....	a 908	2	—	3	—	12	—	4
<i>Fusarium trichothecioides</i> .....	a 941	0	—	0	—	12	—	4
<i>Fusarium martii</i> section.....	268	0	0	4	—	8	—	3
<i>Fusarium</i> between <i>discolor</i> and <i>roseum</i> groups.....	a 946	0	—	4	—	10	—	3
<i>Gibberella saubinetii</i> .....	a 916	—	0	3	0	9	—	3
	a 933	0	—	0	—	0	—	0
	a 915	9	—	0	—	6	—	5
	779	0	4	5	—	0	—	2
	a 913	—	—	—	3	0	—	2
<i>Fusarium elegans</i> group.....	370	0	—	0	—	—	—	0
	736	0	—	0	—	0	—	0
	a 904	0	—	0	—	0	—	0
	a 909	—	—	0	—	—	—	0
	a 912	0	—	—	0	—	—	0
<i>Fusarium culmorum</i> group.....	a 917	0	—	9	—	0	—	3
	a 929	0	—	0	—	—	—	0
	a 557	0	—	0	—	—	—	0
	a 940	—	—	0	0	9	—	3
<i>Fusarium solani</i> .....	a 905	—	—	0	0	—	0	0
	202	0	—	0	—	—	—	0
<i>Fusarium vasinfectum</i> .....	a 928	0	—	5	—	—	—	3
	a 942	0	—	0	—	—	—	0
<i>Fusarium martii</i> .....	a 914	4	0	—	—	—	—	1
<i>Fusarium eumartii</i> .....	a 937	0	—	1	—	0	—	1
<i>Nectria ipomoeae</i> .....	a 931	2	—	0	—	—	—	1
	283	0	—	0	—	0	—	0
<i>Fusarium acuminatum</i> .....	508	0	—	—	0	0	—	0
	a 932	0	—	0	—	—	—	0
	367	—	—	0	0	—	—	0
<i>Fusarium discolor</i> section.....	a 518	0	—	—	—	0	—	0
	935	0	—	0	—	0	—	0
<i>Fusarium ventricosum</i> .....	259	0	—	0	—	—	—	0
<i>Fusarium orthoceras</i> (?).....	357	—	—	0	0	—	—	0
Unidentified <i>Fusarium</i> .....	416	0	0	—	—	—	—	0
Unidentified <i>Fusarium</i> .....	446	—	—	—	—	0	—	0
<i>Fusarium avenaceum</i> .....	a 911	0	—	0	—	—	—	0
<i>Fusarium hyperoxysporum</i> .....	a 930	0	—	0	—	0	—	0
<i>Fusarium coeruleum</i> .....	a 934	0	—	0	—	0	—	0
<i>Fusarium oxysporum</i> .....	a 938	0	0	—	—	—	—	0
<i>Fusarium radicola</i> .....	a 939	—	—	—	0	—	—	0
Average uninoculated unit.....		0	0	0	0	0	.4	.1

\* Lines isolated from nonconiferous hosts.

On the whole, the *Fusarium* lines seemed to cause more decay of radicles of *Pinus resinosa* than of those of *P. banksiana*, but further experiments are necessary to determine whether this difference is constant. A species of *Fusarium* has been reported as causing a blight of maple (6), and others have been listed from such forest-tree seeds as hickory, horsechestnut, and walnut (4).

The results of the inoculation of coniferous radicles with *Fusarium* spp. by the second method confirmed the ability of *F. sporotrichioides* and *F. arthrosporioides* to decay radicles but gave no evidence of such ability for any of the other lines. *F. sporotrichioides* caused 70 per cent of the radicles of *Pinus banksiana* and 50 per cent of those of *Picea engelmanni* to decay, and *F. arthrosporioides* decayed 70 per cent of those of the pine but none of those of the spruce. One line each of the *F. elegans* group and of *Gibberella saubinetii* and two lines of the *F. moniliforme* group decayed no radicles of *Pinus banksiana*. Three lines of the *F. moniliforme* group and one of *G. saubinetii* decayed no radicles of *P. resinosa*. Five lines of the *F. moniliforme* group, two of *G. saubinetii*, two of the *F. elegans* group, one each of the *F. discolor* group, *F. martii*, *F. hyperoxysporum*, and one line of a taxonomic position between the *F. discolor* and *F. roseum* groups were harmless to the radicles of *Picea engelmanni*.

#### BOTRYTIS SPP.

The small-sclerotium type of *Botrytis* (Tables 6 and 8) gave no evidence of ability to decay radicles. The large-sclerotium lines, commonly called *Botrytis cinerea*, were more or less parasitic to young radicles, but appeared far less parasitic to these than to stems (29). In the one experiment in which *Pinus banksiana* radicles were inoculated with line No. 944 by the second method, 30 per cent of the radicles were decayed. *B. cinerea* has been reported as attacking forest-tree seeds such as chestnut (21) and horsechestnut (24). It also causes a disease of pistillate catkins of birch (23).

#### MISCELLANEOUS IMPERFECT FUNGI

Every tested line of *Phomopsis juniperovora* (Tables 7 and 8) caused some radicle decay in every experiment in which it was tested. None of the other tested imperfect fungi showed any evidence of causing radicle decay.

TABLE 6.—Percentages of coniferous radicles decayed by *Botrytis* spp.

Fungus	Line No.	Pinus banksiana			Pinus resinosa, experiment No 134	Picea engelmanni, experiment No. 131	Average
		Experiment No. 100	Experiment No 116	Experiment No. 120			
<i>Botrytis cinerea</i> .....	* 944	22	32	-----	33	0	22
	* 920	10	11	0	0	-----	6
	* 924	10	0	-----	0	-----	4
	470	4	0	-----	2	-----	2
<i>Botrytis</i> spp. (small-sclerotium type).....	* 921	-----	0	0	4	-----	1
	* 922	0	0	-----	0	-----	0
Average uninoculated unit.....	* 923	-----	0	-----	0	-----	0
	-----	0	0	0	0	.4	.1

\* Lines isolated from nonconiferous hosts.

TABLE 7.—Percentages of coniferous radicles decayed by miscellaneous imperfect fungi

Fungus	Line No	Pinus banksiana			Pinus resinosa, experiment No. 134	Average
		Experiment No 100	Experiment No 116	Experiment No. 120		
Phomopsis juniperovora.....	860	38	42	-----	18	35
	864	14	14	-----	81	23
	865	15	12	-----	20	15
	867	11	8	-----	-----	9
Cephalothecium roseum.....	* 283	0	-----	-----	-----	0
Verticillium sp.....	* 399	-----	0	-----	-----	0
Thielavia basicola.....	* 428	0	0	-----	-----	0
Pestalozzia funerea (?).....	968	0	0	0	-----	0
Rhizoctonia potomacensis.....	* 881	0	-----	-----	-----	0
Average uninoculated unit.....	-----	0	0	0	0	0

\* Lines isolated from nonconiferous hosts.

### COMPARISON OF DECAY CAUSED BY THE DIFFERENT FUNGI

A comparison of the average percentages of coniferous radicle decay caused by the different fungi is shown in Table 8.

TABLE 8.—Average percentages of coniferous radicle decay caused by the different fungi

[Fungi listed in alphabetical order. The results for species of which only one line was tested are given in Tables 3, 5, and 7]

Fungi tested	Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average
	Experiment No 100	Experiment No. 110	Experiment No 116	Experiment No 120			
Botrytis spp. (small-sclerotium type).....	0	-----	0	-----	0	-----	0
Botrytis cinerea.....	11	-----	7	0	8	0	7
Coriicium vagum (more virulent lines).	-----	-----	-----	-----	-----	-----	-----
All lines.....	85	50	63	-----	83	25	71
Lines from coniferous hosts.....	78	34	60	-----	68	-----	64
Lines from nonconiferous hosts.....	92	60	96	-----	95	25	79
Line No. 213 and its reisolations.....	95	60	53	-----	97	25	75
Line No. 147 and its reisolations.....	68	34	64	-----	98	-----	65
Line No. 746 and its duplicate.....	96	-----	54	-----	7	-----	55
Coriicium vagum (less virulent lines).	-----	-----	-----	-----	-----	-----	-----
All lines.....	5	7	16	7	10	14	11
Lines from coniferous hosts.....	4	0	14	7	13	-----	9
Lines from nonconiferous hosts.....	7	13	20	-----	7	14	13
Line No. 230 and its reisolations.....	12	13	29	-----	7	14	16
Fusarium spp. (exclusive of No. 906).....	2	1	1	1	6	0	2
Fusarium acuminatum.....	0	-----	0	0	0	-----	0
Fusarium culmorum group.....	0	-----	2	-----	0	-----	1
Fusarium discolor section.....	0	0	0	0	0	-----	0
Fusarium elegans group.....	1	4	1	2	1	-----	1
Fusarium moniliforme species and section.....	8	3	1	2	1	-----	6
Fusarium solani.....	0	-----	0	0	9	0	1
Fusarium vasinfectum.....	0	-----	2	-----	-----	-----	1
Gibberella saubinetii.....	0	0	2	0	4	-----	2
Phomopsis juniperovora.....	19	-----	18	-----	32	-----	21
Phytophthora spp.....	3	-----	32	8	15	-----	15
Pythium aphididermatum.....	74	-----	99	-----	100	-----	90
Pythium artotrogus.....	0	-----	0	-----	0	-----	0
Pythium ultimum:	-----	-----	-----	-----	-----	-----	-----
All lines.....	83	-----	84	98	89	67	82
Lines from coniferous hosts.....	74	-----	79	-----	97	76	81
Lines from nonconiferous hosts.....	90	-----	87	98	85	59	83
Line No. 131 and its reisolations.....	100	-----	97	98	96	54	89
Uninoculated.....	0	0	0	0	0	.4	.1



## SPROUTING DECREASE

The decay of unruptured seeds is, of course, more difficult to determine quantitatively than is the decay of radicles. The only way that it seemed practicable to estimate the amount of seed decay in these experiments was to compare the number of sprouted seeds, indicated by rupturing visible with a hand lens, in each inoculated unit with that in the average uninoculated unit in the same experiment. (Tables 9 to 16.) The normal variability in the germination of coniferous seeds served to introduce a serious source of error in this method of determination. Fortunately, the distributions in the 75 uninoculated units of the three large experiments were practically symmetrical.

In examining the significance of the average differences between inoculated and uninoculated units (next to the last columns in Tables 9 to 15), the procedure used was to determine the probability of an accidental difference in the direction and of the magnitude observed. This was done by the method outlined by Fisher (5, p. 106), halving the probability values given at the top of his table of  $t^7$  (5, p. 137) in accordance with his instructions (5, p. 107). The complement of this halved value was taken as the probability that the observed difference was due to the inoculation. When the difference was an apparent increase in the percentage sprouting, the probability obtained as above was the probability that the inoculation resulted in increased sprouting percentage, and its complement was taken as the probability that the fungus actually decreased germination. In cases of apparent decreases, therefore, the probability that inoculation resulted in decrease (last columns of Tables 9 to 15) is the complement of the probability of accidental occurrence of so large a decrease; for apparent increases it is identical with the probability of accidental occurrence of such an increase.

It seems at first thought impossible to speak of probability of decrease in cases in which there was an apparent increase. As a matter of fact, there is no inconsistency; while the inoculated units in such cases gave higher sprouting percentages than the uninoculated, there is still a chance that the units chosen in advance for inoculation happened to be especially good ones and would have shown a still higher sprouting percentage if they had not been inoculated. Because the available data indicate an increase, there is less than an even chance in such cases that the inoculation caused a decrease, and the probability of decrease is less than 0.5.

The mathematical interpretation can go only far enough to express the probability that the inoculated units under consideration belonged

<sup>7</sup> Fisher's notation is different from that employed in most American literature, particularly in that he uses  $n'$  in place of  $n$ , and  $n$  in place of  $(n-1)$ . The term " $t$ " used by Fisher is the ratio of the mean of a sample to the standard error computed from the sample. His table of  $t$  gives the probability of the mean of a sample departing so far from zero in either the positive or negative direction when the true mean of the population sampled is zero. To use a more common notation, when  $M$  is the mean,  $\sum d^2$  the sum of the squared deviations, and  $n$  the number of items,  $t = \frac{M}{\sqrt{\sum d^2/n(n-1)}}$ . For examining the significance of the difference between means of two samples,  $t$  is the ratio of the difference to its standard error, obtained as follows:

$$t = \frac{M_1 - M_2}{\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right) \frac{\sum d_1^2 + \sum d_2^2}{n_1 + n_2 - 2}}}$$

For a mean based on 10 items, Fisher's table of  $t$  is entered on line 9; for a difference between a mean based on 10 items and a mean based on 8 items, the proper line in the table of  $t$  is  $(10-1) + (8-1)$  or the sixteenth line.

to a population with a lower mean than the uninoculated units. The most logical assumption appears to be that any decreases due to the inoculation were the result of the invasion and killing of the seeds by the fungus added. It is possible, however, that the effect of the inoculation was due to toxins produced by the fungus, to exhaustion of the oxygen in the Petri dish, or to some other indirect influence. The probability computations give no information as to why the inoculated units differed from those uninoculated.

In Tables 9 to 14, inclusive, the lines are arranged in decreasing order of apparent virulence to radicles. The average increase or decrease in sprouting percentage is based on germination in the average uninoculated unit. (Table 1.) The absolute difference between the amount of sprouting in the mean uninoculated unit and that in each individual inoculated unit was calculated; then the percentage sprouting decrease was calculated from this difference and the germination in the mean inoculated unit. The results for the average inoculated units are given in Table 15.

#### PYTHIUM ULTIMUM

The absolute differences between the germination in the mean uninoculated unit and that in the mean unit inoculated with *Pythium ultimum* lines were not great for each experiment, but they were consistent enough to establish the fact that this fungus can kill some seeds which, so far as could be determined externally, had not started to germinate. For the experiments for which probable errors could be calculated, the weighted average difference between the sprouting of the average uninoculated unit and that of the average inoculated unit was  $9.13 \pm 1.05$ .

There was no significant difference between the average amount of reduction in sprouting caused by *Pythium ultimum* lines isolated from coniferous hosts and those from nonconiferous hosts.

Further and more extensive experiments would be necessary to establish definitely the relative virulence of the different lines. As might be expected, the addition of a parasite, an important variable factor, resulted in increasing variability as well as in decreasing sprouting. The coefficients of variability for the number of seeds germinating in the *Pythium ultimum* units were  $18.7 \pm 3.1$  per cent in experiment No. 100,  $11.8 \pm 1.7$  per cent in experiment No. 116, and  $21.4 \pm 3.6$  per cent in experiment No. 134, as compared with  $9.6 \pm 0.92$  per cent,  $11.7 \pm 1.12$  per cent, and  $10.3 \pm 0.98$  per cent for the uninoculated units in the respective experiments. For this reason greater differences in results are required to establish definitely the significance of the differences between the units inoculated with different fungi than between inoculated and uninoculated units.

It is significant that all the average percentage figures in next to the last column of Table 9 are negative and that the probabilities of sproutings being less in inoculated than in uninoculated units are more than 0.80 for all lines except No. 131. The average percentage apparent sprouting decrease for all lines was 17, and for these same lines for all experiments except No. 120, in which there was an unexplained lowering of germination of uninoculated units, the probabilities of sproutings being less in inoculated than in uninoculated units are more than 0.98. (Table 15.)

TABLE 9.—Comparison between the sprouting of coniferous seeds in each unit inoculated with *Pythium ultimum* and that in the average uninoculated unit in the same experiment

Line No.	Apparent increase (+) or decrease (−) in sprouting percentage						Probability that sprouting will be less in inoculated than in uninoculated units
	Pinus banksiana			Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average <sup>a</sup>	
	Experiment No. 100	Experiment No. 116	Experiment No. 120				
810 <sup>b</sup>	−21	−11	—	+14	−16	−9	0.84
550	−14	−36	—	−14	−30	−24	.99
258	−12	−24	—	—	−51	−22	.94
131 <sup>b</sup>	—	+2	−0.9	−29	+7	−5	.71
408 <sup>b</sup> c	−4	−13	—	—	—	−9	.85
338 <sup>b</sup> c	—	−13	+25	−34	−54	−19	.83
295 <sup>b</sup> c	+16	−18	—	−31	−32	−16	.88
767	+3	+0.7	—	−10	−28	−9	.85
296 <sup>b</sup>	−40	−8	—	−44	−51	−36	.98
529 <sup>b</sup>	−29	−22	—	−6	—	−14	.88
743	−23	−18	—	—	−25	−22	.99+

<sup>a</sup> It would probably be preferable to use the geometric means, which would result in greater average decreases; but except in cases of extreme variability, as with line 258, the difference would be slight.

<sup>b</sup> Lines isolated from nonconiferous hosts

<sup>c</sup> Reisolation of line No. 131.

## OTHER PHYCOMYCETES

*Pythium aphanidermatum* and *Pythiacystis citrophthora* caused apparent sprouting decrease in every test with them (Tables 10 and 15), whereas the other Phycomycetes either caused no apparent sprouting decrease or caused it only part of the time.

TABLE 10.—Comparison between the sprouting of coniferous seeds in each unit inoculated with a miscellaneous Phycomycete and that in the average uninoculated unit in the same experiment

Fungus	Line No.	Apparent increase (+) or decrease (−) in sprouting percentage				Average	Probability that sprouting will be less in inoculated than in uninoculated units
		Pinus banksiana			Pinus resinosa, experiment No. 134		
		Experiment No. 100	Experiment No. 116	Experiment No. 120			
Pythium aphanidermatum	{ a 351 b 430	−34 −27	−34 −24		−23	−30 −26	0.99 .98
Pythiaecystis citrophthora	{ a 943 a 843	−21 −4	−3 +8			−12 +2	.80 .39
Phytophthora sp.	{ 358 372	+5 −32	−1		−21 −12	−6 −20	.74 .96
Phytophthora cactorum	{ a 901 778	+14 +7	−24			−5 +7	.50
Pythium artotrogus	{ 821 833	+1 +20	+6		−19	−4 +20	.68
Unidentified Phycomycete	{ a 543 a 927	+14 −		+9	+39	+14 +24	.18
Mucor racemosus							

<sup>a</sup> Lines isolated from nonconiferous hosts.

<sup>b</sup> Reisolation of line No. 351.

<sup>c</sup> Reisolation of line No. 372.

## CORTICIUM VAGUM

Approximately 83 per cent of the *Corticium vagum* lines classified as more virulent to radicles (Table 11) caused some apparent average sprouting decrease, but only a little more than one-third of those for which probabilities could be computed had probabilities of more than 0.80 of the sproutings being less in inoculated than in uninoculated units. The average apparent decrease in sprouting percentage was 9 (Table 15), but the probabilities of sproutings being less in inoculated than in uninoculated units varied greatly for the different experiments. For the experiments for which probable errors could be calculated, the weighted average difference between the sprouting in the average uninoculated unit and that of the average inoculated unit was  $5.6 \pm 0.87$ .

Approximately one-third of the lines of *Corticium vagum* classified as less virulent to radicles (Table 11) caused an apparent average decrease in sprouting, but the variability was so high that only one of these lines had a probability of more than 0.80 of sproutings being less in inoculated than in uninoculated units.

TABLE 11.—Comparison between the sprouting of coniferous seeds in each unit inoculated with *Corticium vagum* and that in the average uninoculated unit in the same experiment

Line No	A pparent increase (+) or decrease (−) in sprouting per- centage						Average	Probabil- ity that sprouting will be less in inoculated than in uninoculated units
	Pinus banksiana				Pinus res- inosa, exper- iment No. 134	Picea engel- manni, exper- iment No. 131		
	Exper- iment No. 100	Exper- iment No. 110	Exper- iment No. 116	Exper- iment No. 120				
More virulent lines:								
183 a	−23		+6		−27		−15	0.86
187 a	−32		−18		−17		−22	.98
213 a	+28		−1		−10	−72	−14	.73
331 a b	+11	+8	−17		−14		−3	.65
340 a b	−19	+19	−1		−10		−3	.63
332 c	+11		−20		+20		+4	.39
333 c	−38		−6		−6		−17	.87
329 c		−16	−13		(?)		−15	.97
147	−8	−34	+20				−7	.65
343 c			−18				−18	
746	−36		+14				−11	.64
747 c d	−25		+30		+7		+4	.41
Less virulent lines:								
240			+8		−16		−4	.61
205	+28		+13		−16		+8	.30
50			−11	+20			+5	.40
341 a e	−19	−16	−13		+3	−11	−11	.98
233 a e	−6	−28	+4		−21	+18	−6	.74
230 a		−23	+27		+3		+2	.45
330 a e	+11		+6		+3	−21	−3	.52
761	−17	−10		+34	−19		−3	.59
724	+5		+8		−6		+2	.34
380	+14		+22				+18	.07
361	+16		+22		+7		+15	.04
362	−21			+32			+6	.43
723				+32			+32	
721			+4				+4	
552			+20				+20	
365	+11		+2				+7	.18
363	+18	−9	+7				+6	.21
189 a	−21		+4				−9	.70
186 a	+20		+7				+10	.24

a Lines isolated from nonconiferous hosts.

b Reisolation of line No. 213.

c Reisolation of line No. 147.

d Duplicate of line No. 746.

e Reisolation or duplicate of line No. 230.

## FUSARIUM SPP.

In general, the *Fusarium* lines caused little apparent sprouting decrease of *Pinus banksiana*. (Table 12.) In some of the units of *P. resinosa* there was decided evidence of sprouting decrease. *Fusarium sporotrichioides* gave decided evidence of ability to cause sprouting decrease in every experiment, fully equaling that of the strongly parasitic *Pythium ultimum* lines. Irrespective of the magnitude of the sprouting decreases, the mere fact that the units inoculated with it always sprouted less than the uninoculated units is evidence that the fungus caused the decrease. A mixed culture of *F. moniliforme* and *F. discolor* also showed decided evidence of ability to decrease sprouting. More than half of the lines belonging to the *moniliforme* section of *Fusarium* have probabilities of more than 0.80 of sproutings being less in the inoculated than in the uninoculated units. The probabilities vary for different experiments. (Table 15.) Eleven other *Fusarium* lines belonging to different species have probabilities of more than 0.80 of sproutings being less than in the inoculated units.

TABLE 12.—Comparison between the sprouting of coniferous seeds in each unit inoculated with *Fusarium*, *Gibberella*, and *Nectria* and that in the average uninoculated unit in the same experiment

Fungus	Line No	Apparent increase (+) or decrease (−) in sprouting percentage						Probability that sprouting will be less in the inoculated than in uninoculated units	
		Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131		Average
		Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120				
<i>Fusarium sporotrichioides</i> .....	α 906	−23	−40	−36	—	−34	−70	−41	0.99+
<i>Fusarium discolor sulphureum</i> .....	α 936	−2	—	−11	—	—	—	−7	.82
	α 948	−17	—	−6	—	−23	—	−15	.95
	247	+11	—	−11	—	—	—	0	—
	266	−8	—	—	+46	−14	—	+8	.36
	α 949	+5	—	−13	—	−27	—	−12	.84
	α 308	−17	—	+7	—	−14	—	−10	.89
<i>Fusarium moniliforme</i> species and section.....	α 910	−16	—	−5	—	−16	—	−12	.96
	273	−6	−3	+8	—	—	—	−3	.53
	α 947	−27	—	−6	—	−8	—	−14	.92
	239	—	—	+11	+25	−27	—	+3	.43
	249	−4	−28	—	+23	−48	—	−14	.79
	251	—	—	+13	+44	−6	—	+17	.18
	260	−6	—	+2	—	−46	—	−17	.82
	265	−2	—	−18	—	−10	—	−10	.92
<i>Fusarium arthrosporioides</i> .....	α 908	−4	—	+2	—	−53	—	−18	.80
<i>Fusarium trichothecioides</i> .....	α 941	−27	—	−3	—	−21	—	−17	.93
<i>Fusarium martii</i> section.....	268	−2	−32	−11	—	−6	—	−11	.89
<i>Fusarium</i> between <i>discolor</i> and <i>roseum</i> groups.....	α 946	+1	—	−6	—	−42	—	−16	.82
<i>Gibberella saubinetii</i> .....	α 916	—	−23	+7	−3	−17	—	−11	.93
	α 933	+9	—	+18	—	−2	—	+8	.15
	α 915	−19	—	−13	—	−10	—	−14	.98
	779	−17	−1	−1	—	−38	—	−14	.87
	α 913	—	—	—	+18	−14	—	+2	.46
<i>Fusarium elegans</i> group.....	370	+11	—	−5	—	—	—	+3	.39
	736	+22	—	+8	—	−6	—	+10	.14
	α 904	+9	—	−6	—	−6	—	−2	.57
	α 909	—	—	+8	+27	—	—	+18	.15
	α 912	−6	—	—	+13	—	—	+4	.27
	α 917	−6	—	−18	—	−29	—	−16	.91
<i>Fusarium culmorum</i> .....	α 923	+13	—	−11	—	—	—	+1	.48
	557	+5	—	+15	—	—	—	+10	.15
	α 940	—	—	−3	+14	−17	—	−7	.68
<i>Fusarium solani</i> .....	α 905	—	—	+8	+37	—	−44	−3	.50
	202	+39	—	+11	—	—	—	+25	.17
<i>Fusarium vasinfectum</i> .....	α 928	+5	—	+8	—	—	—	+7	.08
	α 942	+7	—	+18	—	—	—	+13	.13

α Lines isolated from nonconiferous hosts.

TABLE 12.—*Comparison between the sprouting of coniferous seeds in each unit inoculated with Fusarium, Gibberella, and Nectria and that in the average uninoculated unit in the same experiment—Continued*

Fungus	Line No.	Apparent increase (+) or decrease (–) in sprouting percentage						Average	Probability that sprouting will be less in the inoculated than in uninoculated units
		Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131		
		Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120				
<i>Fusarium martii</i> .....	a 914	+7	–3	+0.7				+2	0.28
<i>Fusarium eumartii</i> .....	a 937	–6		+16		+3		+4	.30
<i>Nectria ipomoeae</i> .....	a 931	–4		+6				+1	.43
	283	+11		–13		–16		–6	.72
<i>Fusarium acuminatum</i> .....	a 508	+7			+32	–29		+3	.44
	a 932	+16		–1				+8	.26
	367			+2	+25			+14	.22
<i>Fusarium discolor</i> section.....	a 518	+11		–13		+3		–1	.54
	a 935	+5		+13		+22		+13	.05
<i>Fusarium ventricosum</i> .....	299	+18		–22				–2	.54
<i>Fusarium orthoceras</i> (?).....	357			–5	+46			+21	.20
Unidentified <i>Fusarium</i> .....	416	–6	–17	–3				–7	.85
Unidentified <i>Fusarium</i> .....	446	+22				–10		+6	.39
<i>Fusarium avenaceum</i> .....	a 911	+9		+7				+5	.32
<i>Fusarium hyperoxysporum</i> .....	a 930	–6		+6		+7		+4	.12
<i>Fusarium coeruleum</i> .....	a 934	+22		+4		+24		+17	.06
<i>Fusarium oxysporum</i> .....	a 938	+36	–28	+13				+7	.37
<i>Fusarium radicola</i> .....	a 939			–15	+13			–1	.53
Average inoculated unit exclusive of line No. 906.....		+3	–17	–1	+25	–15	–44	–1	

a Lines isolated from nonconiferous hosts.

## BOTRYTIS SPP.

The small-sclerotium type of *Botrytis* and three lines of *Botrytis cinerea* caused no apparent average decrease in sprouting percentage. (Table 13.) The other two lines of *B. cinerea* not only caused a large apparent average decrease in sprouting percentage but also had high probabilities of sproutings being less in inoculated than in uninoculated units. In three experiments (Table 15) there were high probabilities of sproutings being less in the inoculated units.

TABLE 13.—*Comparison between the sprouting of coniferous seeds in each unit inoculated with Botrytis spp. and that in the average uninoculated unit in the same experiment*

Fungus	Line No.	Apparent increase (+) or decrease (–) in sprouting percentage						Average	Probability that sprouting will be less in inoculated than in uninoculated units
		Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131		
		Experiment No. 100	Experiment No. 116	Experiment No. 120					
<i>Botrytis cinerea</i> .....	a 914	–17	–34			–16	–33	–25	0.99
	a 920	–44	–1	+4		–72		–28	.89
	a 924	+11	–8			+7		+3	.33
	470	–6	–5			+7		+4	.46
<i>Botrytis</i> spp. (small-sclerotium form).....	a 921		+13	+75		–6		+27	.20
	a 922	+26	+9			–25		+3	.43
	a 923		–1			+11		+5	.28

a Lines isolated from nonconiferous hosts.

## MISCELLANEOUS IMPERFECT FUNGI

*Phomopsis juniperovora* caused considerable apparent sprouting decrease and had high probabilities of sprouting's being less in the inoculated than in the uninoculated units. (Tables 14 and 15.) *Cephalothecium roseum*, *Verticillium* sp., *Pestalozzia funerea* (?), and *Rhizoctonia potomacensis* all caused some apparent sprouting decrease.

TABLE 14.—Comparison between the sprouting of coniferous seeds in each unit inoculated with miscellaneous imperfect fungi and that in the average uninoculated unit in the same experiment

Fungus	Line No.	Apparent increase (+) or decrease (–) in sprouting percentage					Probability that sprouting will be less in inoculated than in uninoculated units
		Pinus banksiana			Pinus resinosa, experiment No. 134	Average	
		Experiment No. 100	Experiment No. 116	Experiment No. 120			
Phomopsis juniperovora	860	–12	–17	-----	–47	–25	0.93
	864	–17	–11	-----	–70	–33	.89
	865	+3	–15	-----	–34	–15	.85
	867	+1	–8	-----	-----	–4	.74
Cephalothecium roseum	* 288	–32	-----	-----	-----	–32	-----
Verticillium sp.	* 399	-----	–10	-----	-----	–10	-----
Thielavia basicola	* 428	–12	+15	-----	-----	+2	.45
Pestalozzia funerea (?)	968	–19	–8	+18	-----	–3	.59
Rhizoctonia potomacensis	* 881	–6	-----	-----	-----	–6	-----

\* Lines isolated from nonconiferous hosts.

## COMPARISON OF SPROUTING DECREASES CAUSED BY THE DIFFERENT FUNGI

A comparison of the sprouting decreases caused by the different fungi is shown in Table 15.

TABLE 15.—*Comparison between the sprouting of coniferous seeds in the average inoculated unil and that in the average uninoculated unil*

[illegible]



## RELATION BETWEEN RADICLE DECAY AND SPROUTING DECREASE

Table 16 summarizes the relationship between radicle decay and sprouting decrease. With the exception of the less virulent lines of *Corticium vagum*, in more than 70 per cent of the units radicle decay was accompanied by apparent sprouting decrease.

However, for both *Pythium ultimum* and *Corticium vagum* the lines that caused the most apparent decrease in sprouting were not always the ones that decayed the most radicles. *Pythium aphanidermatum*, *Pythiacystis citrophthora*, and *Fusarium sporotrichioides* caused apparent sprouting decrease and radicle decay in every test with them.

*Botrytis cinerea*, line No. 944, which seemed most virulent to young radicles, caused some apparent sprouting decrease in each of the four experiments in which it was tested. Line No. 920, which was the only other one that showed any definite ability to decay radicles, apparently decreased sprouting in three out of the four experiments in which it was used. Seven of the eight units inoculated with these two lines gave lower germination than the uninoculated units. Irrespective of the magnitude of the differences, their uniformity of direction constitutes decided evidence that they are not accidental. In the experiments with numerous uninoculated units, and hence with relatively reliable basis for comparison, all the units inoculated with these two lines germinated less well than the uninoculated units. These differences expressed in terms of the probable error of a single uninoculated unit were 6.7, 2.7, 0.1, 4.3, 10.3, 2.2, and 5.3. Thus, the evidence is rather strong, though not conclusive because of the small number of tests, that the lines of *Botrytis cinerea* capable of decaying radicles were also capable of causing sprouting decrease.

TABLE 16.—Relation between radicle decay and sprouting decrease caused by fungi in certain coniferous seeds during germination

Inoculating fungus	Units with radicle decay		Units without radicle decay	
	Number	Percentage in which there was apparent sprouting decrease	Number	Percentage in which there was apparent sprouting decrease
<i>Pythium aphanidermatum</i> .....	5	100	0	—
<i>Pythiacystis citrophthora</i> .....	2	100	0	—
<i>Pythium ultimum</i> .....	40	82	0	—
<i>Phomopsis juniperovora</i> .....	11	82	0	—
<i>Botrytis cinerea</i> .....	9	78	8	50
<i>Fusarium</i> spp. ....	44	75	109	51
<i>Phytophthora</i> spp. ....	7	72	3	67
More virulent lines of <i>Corticium vagum</i> .....	35	71	0	—
Less virulent lines of <i>Corticium vagum</i> .....	30	47	20	25
<i>Botrytis</i> spp. (small-sclerotium type).....	0	—	5	40
Other fungi.....	0	—	16	44

*Phomopsis juniperovora* and *Phytophthora* sp. appeared to cause sprouting decrease in some units of *Pinus banksiana*, and in the one experiment with *P. resinosa* they decreased sprouting in all units inoculated with them. One unit of *P. banksiana* inoculated with *Cephalothecium roseum* showed no rotted radicles, but the germination was significantly lower than that in the average uninoculated

unit. In this case the germination differed from the mean of the uninoculated units by about five times the probable error of a single uninoculated unit. It seems, therefore, reasonable to believe that the addition of the *Cephalothecium* was somewhat effective, making the unit essentially different from the average uninoculated unit. However, despite the symmetrical distribution of sprouting percentages in the uninoculated units of all three large experiments, there was an unexplained lowering in the sprouting percentage of three of the uninoculated plates in the small experiment No. 120, which makes it appear that differences can not be interpreted strictly in terms of their probable errors, even in the larger experiments; and the data on this one plate, therefore, by no means establish the ability of *Cephalothecium* to cause seed decay. Further experimentation is necessary to substantiate this suggestion.

Most of the *Fusarium* and *Corticium* lines that were less virulent to young radicles, most of the *Botrytis cinerea* lines, the small sclerotium *Botrytis* lines, and the remaining fungi showed no appreciable apparent ability to decay unruptured seeds of *Pinus banksiana*. However, it is evident, from a consideration of the figures for *P. banksiana*, that with sprouting taking place as quickly as it did in these experiments (approximately two weeks after planting) the decay of unruptured seeds is a less important part of germination loss than the decay of radicles shortly after rupture of the seed coats. It is also obvious that the only fungi able to cause significant rotting of unruptured seeds were those able to attack radicles.

For *Pinus resinosa* the losses both before and after sprouting were, on the whole, about the same as for *P. banksiana*, but they were distributed differently, furnishing less support for correlation between ability to decay radicles soon after sprouting and that to cause sprouting decrease. *Fusarium* lines, for example, which caused no decay of radicles, apparently decreased the sprouting percentages in *P. resinosa*.

In the spruce experiment, in which there were only six uninoculated units, but these in excellent agreement with one another, there was distinct indication of seed decay by most of the fungi used, including many that had no effect at all on the radicles of the seed that sprouted. Because of the small number of units, especially of the uninoculated ones, great weight can not be attached to the results with this species.

The fact that the fungi able to attack radicles were also, in general, the ones able to decrease sprouting raises the question whether the seeds whose germination was prevented were really decayed while still truly dormant. It seems reasonable to believe, in view of the apparent relation between the ability of the various fungi to rot radicles and to prevent the germination of unruptured seed, that the seeds whose sprouting was prevented were attacked after the changes preceding germination had gone so far inside the seeds that they could no longer be considered truly dormant. It is, of course, also possible that some of the seed reported as unruptured may have really been ruptured but immediately killed by the penetration of hyphae of the parasite before the rupture of the seed coat had proceeded far enough to be detected by a rapid examination with a hand lens. Whatever the truth may be as to this, it is evident, from experiments in which the fungus enjoyed very favorable conditions for its

spread into the seed, that when germination is prompt such seed decay as occurs in the seed bed is likely to be of minor importance and caused chiefly by damping-off fungi. Where germination loss is heavy, most of the loss may usually be attributed to the infection of seeds that have already sprouted by damping-off fungi.

Attention has been called by some writers (9, 12, 14, 17, 19, 31) to the fact that old, injured, stored, or slowly germinating seeds may be decayed by saprophytic organisms; but, as others (15, 16, 33) have stated that coniferous seeds germinate after storage for several years in the forest floor, it is probable that really dormant seeds are not seriously decayed by fungi other than damping-off parasites. However, in an unpublished note Hartley reported that in some laboratory germination tests with *Pseudotsuga taxifolia*, *Trichoderma*, *Mucor*, and *Penicillium*, but no *Pythium*, *Rhizoctonia*, or *Botrytis*, were present in the seeds that failed to germinate.

#### SUMMARY

Experiments in which coniferous seeds were germinated in contact with agar cultures of fungi appear to warrant the following statement: Poor germination of pine, as a result of the work of damping-off parasites, is mainly due to the destruction of radicles after they have emerged from the seed coat but before the seedlings are large enough to break through the soil. Decay of unruptured seed apparently can be caused by these fungi, but it appears to be of less importance as a cause of germination loss.

*Pythium ultimum*, *P. aphanidermatum*, *Pythiacystis citrophthora*, *Phytophthora* spp., *Fusarium sporotrichioides*, *F. discolor sulphureum*, *F. arthrosporioides*, *Botrytis cinerea*, *Phomopsis juniperovora*, and some lines of *Corticium vagum* and *F. moniliforme* were able to decay radicles that had just emerged from the seed coats. Several other species of *Fusarium* attacked recently emerged radicles at times, but not frequently enough to establish either their parasitic or their nonparasitic ability.

The following fungi failed to attack radicles under the conditions of these experiments: *Cephalothecium roseum*, *Verticillium* sp., *Thielavia basicola*, *Pestalozzia funerea* (?), the small sclerotial *Botrytis* spp., and some of the *Corticium vagum* lines, *Fusarium radicicola*, *F. oxysporum*, *F. coeruleum*, *F. hyperoxysporum*, *F. avenaceum*, *F. orthoceras* (?), *F. ventricosum*, three lines of *F. acuminatum*, three lines belonging to the *discolor* section of *Fusarium*, *Pythium artotrogus*, *Mucor racemosus*, and an unidentified Phycomycete.

All the available lines of *Pythium ultimum* and some of the more virulent lines of *Corticium vagum* gave apparent evidence of attacking seeds that had not ruptured, so far as could be detected with a hand lens. *P. aphanidermatum*, *Pythiacystis citrophthora*, *Fusarium sporotrichioides*, and *Phomopsis juniperovora*, none of which have been isolated from damped-off coniferous seedlings under natural conditions, also appeared to attack unruptured seeds. Several of the other fungi gave less apparent evidence of this ability.

The results in the one experiment with *Pinus resinosa* agreed in general with those of *P. banksiana*, except that the seeds of *P. resinosa* showed reduced sprouting percentages with *Fusarium* lines and with certain other fungi that failed to attack radicles.

In a preliminary experiment *Picea engelmanni* appeared more susceptible to seed decay than either of the pines.

*Pythium ultimum* and the more virulent lines of *Corticium vagum*, in the experiments reported, caused more apparent germination loss than any tested species of *Fusarium* except *F. sporotrichioides*.

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# GERMICIDAL EFFICIENCY OF SODIUM HYDROXIDE, SODIUM CARBONATE, AND TRISODIUM PHOSPHATE<sup>1</sup>

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## INTRODUCTION

In this paper the writers present results which they obtained, for the most part, in making a comparative study of disinfectants that might be used by poultrymen, a fact which accounts for the choice of test organisms and various other details of the technic. Although, as the title implies, this work was done chiefly with the hydroxide, carbonate, and phosphate of sodium, the paper also reports the results of tests with various commonly used disinfectants.

## EXPERIMENTAL PROCEDURE

The solutions of sodium hydroxide used for the bacteriological tests were made from standardized carbonate-free stock solutions already prepared for chemical work; the solutions of sodium carbonate were made from the anhydrous chemically pure salt; and the solutions of trisodium phosphate were made from the crystalline chemically pure salt containing 12 molecules of water, concentrations being given in the tables on a basis of trisodium phosphate without regard to water. The calcium hydroxide used in some of the experiments was usually prepared by hydration of United States Pharmacopoeia calcium oxide, but in some instances a high-grade commercial sample of hydrate of lime was employed.

Bacteriological tests were made by the Rideal-Walker technic, modified as described in a previous paper published in this journal,<sup>2</sup> and also by tests made at various medication temperatures instead of at a uniform temperature of 25° C.

## RESULTS OF EXPERIMENTS

The results of bacteriological tests with sodium hydroxide against *Eberthella typhi*<sup>3</sup> (*Bacillus typhosus*), *Staphylococcus aureus*, *Salmonella pullorum*, *Pasteurella avicida*, *Salmonella gallinarum*, and two strains of hemolytic streptococci are shown in Table 1. In general, the results indicate that sodium hydroxide is an efficient germicide against these organisms even in the presence of organic matter in the form of skim milk.

<sup>1</sup> Received for publication Sept. 23, 1930; issued January, 1931.

<sup>2</sup> SCHAFFER, J. M., and TILLEY, F. W. GERMICIDAL EFFICIENCY OF SOAPS AND OF MIXTURES OF SOAPS WITH SODIUM HYDROXIDE OR WITH PHENOLS. Jour. Agr. Research 41: 737-747. 1930.

<sup>3</sup> The bacteriological nomenclature used in this paper is that of the following publication: BERGEY, D. H. BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY; A KEY FOR THE IDENTIFICATION OF ORGANISMS OF THE CLASS SCHIZOMYCETES \* \* \* Ed. 3, 589 p., Baltimore. 1930.

TABLE 1.—*Germicidal efficiency of sodium hydroxide against Eberthella typhi<sup>1</sup> Staphylococcus aureus, and other test organisms<sup>a</sup>*

Disinfectant	Dilution	Organic matter	Growth <sup>b</sup> after indicated period of exposure (minutes)						Test organism
			2½	5	7½	10	12½	15	
Phenol.....	1-100	None.....	+	+	-	-	-	-	E. typhi No. 1.
NaOH.....	1-1, 400	do.....	+	+	-	-	-	-	Do.
Do.....	1-1, 600	do.....	+	+	+	-	-	-	Do.
Do.....	1-1, 800	do.....	+	+	+	+	-	-	Do.
Do.....	1-300	Skim milk, 50 per cent.	-	-	-	-	-	-	Do.
Do.....	1-400	do.....	+	+	-	-	-	-	Do.
Do.....	1-500	do.....	+	+	+	+	+	+	Do.
Phenol.....	1-95	None.....	+	+	+	-	-	-	E. typhi No. 2.
NaOH.....	1-1, 200	do.....	+	+	+	-	-	-	Do.
Do.....	1-1, 600	do.....	+	+	+	+	+	+	Do.
Phenol.....	1-70	do.....	+	+	+	-	-	-	Staph. aureus.
NaOH.....	1-80	do.....	+	+	+	-	-	-	Do.
Do.....	1-100	do.....	+	+	+	-	-	-	Do.
Do.....	1-120	do.....	+	+	+	+	-	-	Do.
Do.....	1-50	Skim milk, 50 per cent.	+	+	+	-	-	-	Do.
Do.....	1-60	do.....	+	+	+	+	-	-	Do.
Do.....	1-70	do.....	+	+	+	+	+	+	Do.
Phenol.....	1-90	None.....	+	+	+	-	-	-	Salmonella pullorum.
NaOH.....	1-1, 200	do.....	+	+	+	-	-	-	Do.
Do.....	1-1, 600	do.....	+	+	+	+	+	+	Do.
Do.....	1-300	Skim milk, 50 per cent.	+	+	+	-	-	-	Do.
Do.....	1-400	do.....	+	+	+	+	+	+	Do.
Phenol.....	1-160	None.....	+	+	+	-	-	-	Pasteurella avicida.
NaOH.....	1-1, 600	do.....	+	+	+	-	-	-	Do.
Do.....	1-2, 000	do.....	+	+	+	-	-	-	Do.
Do.....	1-400	Skim milk, 50 per cent.	+	+	+	-	-	-	Do.
Do.....	1-500	do.....	+	+	+	+	-	-	Do.
Phenol.....	1-100	None.....	+	+	+	-	-	-	Salmonella gallinarum.
NaOH.....	1-1, 200	do.....	+	+	+	-	-	-	Do.
Do.....	1-1, 600	do.....	+	+	+	+	+	+	Do.
Do.....	1-300	Skim milk, 50 per cent.	+	+	+	-	-	-	Do.
Do.....	1-400	do.....	+	+	+	+	+	+	Do.
NaOH.....	1-100	None.....	+	+	+	+	+	+	Do.
Do.....	1-150	do.....	+	+	+	+	+	+	Streptococcus No. 1.
Do.....	1-600	do.....	+	+	+	+	+	+	Do.
Do.....	1-800	do.....	+	+	+	+	+	+	Streptococcus No. 2.
									Do.

<sup>a</sup> Experiments conducted at 25° C.<sup>b</sup> + indicates growth, —, no growth.TABLE 2.—*Germicidal efficiency of various disinfectants against Salmonella pullorum<sup>a</sup>*

Disinfectant	Dilution	Organic matter	Growth <sup>b</sup> after indicated period of exposure (minutes)					
			2½	5	7½	10	12½	15
Phenol.....	1-90	None.....	+	+	-	-	-	-
Formalin.....	1-20	do.....	+	+	-	-	-	-
Do.....	1-40	do.....	+	+	+	-	-	-
Do.....	1-60	do.....	+	+	+	+	+	+
Do.....	1-30	Skim milk, 50 per cent.	+	+	+	+	+	+
Do.....	1-40	do.....	+	+	+	+	+	+
Cresol, U. S. P.....	1-240	None.....	+	+	+	+	-	-
Do.....	1-280	do.....	+	+	+	+	-	-
Do.....	1-260	Skim milk, 50 per cent.	+	+	+	+	-	-
Do.....	1-220	do.....	+	+	+	+	+	+
Liquor cresolis compositus, U. S. P.....	1-160	None.....	+	-	-	-	-	-
Do.....	1-200	do.....	+	+	+	-	-	-
Do.....	1-240	do.....	+	+	+	+	+	-
Do.....	1-120	Skim milk, 50 per cent.	+	+	+	+	+	-
Do.....	1-140	do.....	+	+	+	+	+	+
Lime water.....		None.....	+	-	-	-	-	-
Milk of lime.....		Skim milk, 50 per cent.	+	-	-	-	-	-
NaOH.....	1-1, 600	None.....	+	+	+	-	-	-
Do.....	1-400	Skim milk, 50 per cent.	+	+	+	-	-	-

<sup>a</sup> Experiments conducted at 25° C.<sup>b</sup> + indicates growth; —, no growth.

Experiments were next made with the same technic to ascertain the efficiency of various other disinfectants as compared with that of sodium hydroxide. The results of these comparative tests are shown in Table 2. Then in order to test all the disinfectants under conditions more closely approximating those met in practice, experiments were made with organic matter in the form of chicken feces, sterilized in the autoclave to avoid complications from bacteria already present, 1 gram of feces to 5 c. c. of disinfectant being used. The results, shown in Table 3, indicate that a 1 per cent solution of sodium hydroxide is effective, in exposures of 5 minutes or more, against all the three test organisms except *Staphylococcus aureus*, which requires a 3 per cent solution.

TABLE 3.—Germicidal efficiency against various organisms <sup>a</sup> of sodium hydroxide and other disinfectants in the presence of chicken feces <sup>b</sup>

Disinfectant	Dilution	Growth <sup>c</sup> after indicated period of exposure (minutes)			Test organism
		5	10	15	
Phenol.....	1- 80	+	-	-	Salmonella pullorum.
Cresol U S P.....	1-160	-	-	-	Do.
Do.....	1-300	+	+	-	Do.
Liquor cresolis compositus U. S P.....	1-100	-	-	-	Do.
Do.....	1-150	+	+	+	Do.
Formalin.....	1- 20	-	-	-	Do.
Do.....	1- 30	+	-	-	Do.
Milk of lime.....	.....	-	-	-	Do.
NaOH.....	1-100	-	-	-	Do.
Do.....	1-200	+	+	+	Do.
Do.....	1-200	-	-	-	Pasteurella avicida
Do.....	1-300	+	-	-	Do.
Do.....	1-400	+	+	+	Do.
Do.....	1-200	-	-	-	Salmonella gallinarum.
Do.....	1-300	+	+	+	Do.
Do.....	1- 33	-	-	-	Staphylococcus aureus.
Do.....	1- 50	+	-	-	Do.
Do.....	1-100	+	+	+	Do.

<sup>a</sup> Experiments conducted at 25° C.

<sup>b</sup> 1 gm. feces to 5 c. c. disinfectant.

<sup>c</sup> + Indicates growth; -, no growth.

A study was then made of the germicidal efficiency of sodium carbonate and of mixtures containing sodium carbonate and sodium hydroxide. The results obtained are shown in Tables 4, 5, 6, and 7. Table 5 also shows results obtained with mixtures containing sodium hydroxide and sodium chloride.



TABLE 4.—Germicidal efficiency of sodium carbonate against *Salmonella pullorum*

EXPERIMENT 1

Na <sub>2</sub> CO <sub>3</sub> dilution	Organic matter	Growth <sup>a</sup> after indicated period of exposure (minutes)						Medica- tion tem- perature
		2½	5	7½	10	12½	15	
1 to 5.....	None.....	+	+	+	+	+	+	° C. 25
1 to 10.....	do.....	+	+	+	+	+	+	25
1 to 20.....	do.....	+	+	+	—	—	—	25
1 to 40.....	do.....	+	+	—	—	—	—	25
1 to 80.....	do.....	+	+	+	+	—	—	25

EXPERIMENT 2

		15	30	45	60	90	120	
1 to 100.....	None.....	+	—	—	—	—	—	° C. (b) 40
1 to 200.....	do.....	+	+	+	+	+	+	(b) 40
1 to 300.....	do.....	+	+	—	—	—	—	(b) 40
1 to 400.....	do.....	+	+	—	—	—	—	40
1 to 500.....	do.....	+	+	—	—	—	—	40
1 to 600.....	do.....	+	+	—	—	—	—	50
1 to 800.....	do.....	+	+	—	—	—	—	50
1 to 1,200.....	do.....	+	+	—	—	—	—	50
1 to 1,600.....	do.....	+	+	—	—	—	—	50
1 to 2,000.....	do.....	+	+	—	—	—	—	50
( <sup>d</sup> ).....	Distilled water.....	+	+	+	+	+	+	50

EXPERIMENT 3

		2½	5	7½	10	12½	15	
1 to 40.....	Skim milk, 50 per cent.....	+	—	—	—	—	—	° C. 40
1 to 60.....	do.....	+	+	+	—	—	—	40
1 to 80.....	do.....	+	+	+	+	—	—	40
1 to 100.....	do.....	+	+	+	+	+	—	40
1 to 80.....	do.....	—	—	—	—	—	—	50
1 to 120.....	do.....	—	—	—	—	—	—	50
1 to 160.....	do.....	+	—	—	—	—	—	50
1 to 200.....	do.....	+	+	—	—	—	—	50
1 to 240.....	do.....	+	+	—	—	—	—	50
1 to 280.....	do.....	+	+	+	+	+	+	50

<sup>a</sup> + indicates growth; —, no growth.  
<sup>b</sup> Room temperature, between 22° and 32° C.  
<sup>c</sup> Growth after 5 hours.  
<sup>d</sup> No Na<sub>2</sub>CO<sub>3</sub> used.

TABLE 5.—Germicidal efficiency of sodium hydroxide and sodium carbonate or sodium chloride against *Salmonella pullorum*

Na <sub>2</sub> CO <sub>3</sub> or NaCl	Dilution	NaOH dilution	Growth <sup>a</sup> after indicated period of exposure (minutes)										Medication temperature
			5	10	15	30	45	60	90	120	150	300	
(b).....		1-1,000	—	—	—	—	—	—	—	—	—	—	° C.
(b).....		1-1,500	+	+	+	—	—	—	—	—	—	—	(°)
(b).....		1-2,000	+	+	+	+	+	+	+	—	—	—	(°)
(b).....		1-2,500	+	+	+	+	+	+	+	+	+	+	(°)
Na <sub>2</sub> CO <sub>3</sub> .....	1-200	1-4,000	+	+	+	—	—	—	—	—	—	—	(°)
Do.....	1-200	1-8,000	+	+	+	+	+	—	—	—	—	—	(°)
Do.....	1-200	1-12,000	+	+	+	+	+	+	—	—	—	—	(°)
Do.....	1-600	1-1,500	+	+	—	—	—	—	+	—	—	—	(°)
Do.....	1-600	1-2,000	+	+	+	—	—	—	—	—	—	—	(°)
Do.....	1-600	1-2,500	+	+	+	+	—	—	—	—	—	—	(°)
Do.....	1-200	1-1,500	+	+	—	—	—	—	—	—	—	—	(°)
Do.....	1-200	1-2,000	+	+	+	+	—	—	—	—	—	—	(°)
NaCl.....	1-200	1-2,500	+	+	+	+	+	+	+	+	—	—	(°)
(b).....		1-2,000	+	+	+	—	—	—	—	—	—	—	40
(b).....		1-3,000	+	+	+	+	+	+	+	+	—	—	40
Na <sub>2</sub> CO <sub>3</sub> .....	1-800	1-2,000	+	+	+	+	+	+	—	—	—	—	40
Do.....	1-800	1-4,000	+	+	+	+	+	+	—	—	—	—	40
Do.....	1-800	1-6,000	+	+	+	+	+	+	—	—	—	—	40
Do.....	1-200	1-2,000	+	+	+	+	+	+	—	—	—	—	40
NaCl.....	1-200	1-3,000	+	+	+	+	+	+	+	+	—	—	40
(b).....		1-2,000	—	—	—	—	—	—	—	—	—	—	50
(b).....		1-3,000	+	+	+	—	—	—	—	—	—	—	50
(b).....		1-4,000	+	+	—	+	+	—	—	—	—	—	50
Na <sub>2</sub> CO <sub>3</sub> .....	1-2,000	1-4,000	+	+	—	—	—	—	—	—	—	—	50
NaCl.....	1-200	1-3,000	+	+	—	—	—	—	—	—	—	—	50
Do.....	1-200	1-4,000	+	+	+	+	+	—	—	—	—	—	50

<sup>a</sup> + indicates growth; —, no growth.<sup>c</sup> Room temperature, between 22° and 32° C.<sup>b</sup> No Na<sub>2</sub>CO<sub>3</sub> nor NaCl usedTABLE 6.—Germicidal efficiency of sodium hydroxide and sodium carbonate against *Salmonella pullorum* with organic matter present

Na <sub>2</sub> CO <sub>3</sub> dilution	NaOH dilution	Organic matter	Growth <sup>a</sup> after indicated period of exposure (minutes)						Medication temperature
			5	10	15	30	45	60	
1 to 50.....	1-400	Skim milk, 50 per cent.....	—	—	—	—	—	—	° C.
Do.....	1-600	do.....	+	+	—	—	—	—	(b)
Do.....	1-800	do.....	+	+	—	—	—	—	(b)
1 to 100.....	1-400	do.....	—	—	—	—	—	—	(b)
Do.....	1-600	do.....	+	+	—	—	—	—	(b)
Do.....	1-800	do.....	+	+	+	—	—	—	(b)
1 to 200.....	1-400	do.....	—	—	—	—	—	—	(b)
Do.....	1-600	do.....	+	+	+	—	—	—	(b)
Do.....	1-800	do.....	+	+	+	+	+	—	(b)
Do.....	1-500	do.....	+	+	—	—	—	—	40
Do.....	1-1,000	do.....	+	+	+	—	—	—	40
Do.....	1-2,000	do.....	+	+	+	—	—	—	40
1 to 400.....	1-400	do.....	+	—	—	—	—	—	40
Do.....	1-600	do.....	+	+	—	—	—	—	40
Do.....	1-800	do.....	+	+	+	—	—	—	40
1 to 800.....	1-1,000	do.....	—	—	—	—	—	—	50
Do.....	1-1,500	do.....	+	+	+	—	—	—	50
Do.....	1-2,000	do.....	+	+	+	+	—	—	50
Do.....	1-3,000	do.....	+	+	+	+	+	—	50

<sup>a</sup> + indicates growth, —, no growth.<sup>b</sup> Room temperature, between 22° and 32° C.

TABLE 7.—*Germicidal efficiency against Staphylococcus aureus of sodium hydroxide and sodium carbonate without and with organic matter present*

Na <sub>2</sub> CO <sub>3</sub> dilution	NaOH dilution	Organic matter	Growth <sup>a</sup> after indicated period of exposure (minutes)						Medication temperature °C
			5	10	15	30	45	60	
1 to 50	( <sup>b</sup> )	None	+	+	+	+	+	+	25
( <sup>c</sup> )	1-100	do	+	+	+	+	+	+	25
( <sup>d</sup> )	1-150	do	+	+	+	+	+	+	25
1 to 100	1-100	do	+	+	+	+	+	+	25
Do	1-200	do	+	+	+	+	+	+	25
Do	1-400	do	+	+	+	+	+	+	25
1 to 5	( <sup>b</sup> )	do	+	+	+	+	+	+	40
1 to 40	( <sup>b</sup> )	do	+	+	+	+	+	+	40
( <sup>d</sup> )	1-400	do	+	+	+	+	+	+	40
( <sup>d</sup> )	1-800	do	+	+	+	+	+	+	40
1 to 50	1-400	do	+	+	+	+	+	+	40
Do	1-800	do	+	+	+	+	+	+	40
Do	1-1,600	do	+	+	+	+	+	+	40
1 to 100	1-400	do	+	+	+	+	+	+	40
Do	1-800	do	+	+	+	+	+	+	40
Do	1-1,600	do	+	+	+	+	+	+	40
1 to 200	1-400	do	+	+	+	+	+	+	40
Do	1-800	do	+	+	+	+	+	+	40
Do	1-1,200	do	+	+	+	+	+	+	40
Do	1-1,600	do	+	+	+	+	+	+	40
1 to 5	( <sup>b</sup> )	do	+	+	+	+	+	+	50
1 to 40	( <sup>b</sup> )	do	+	+	+	+	+	+	50
( <sup>d</sup> )	1-400	do	+	+	+	+	+	+	50
( <sup>d</sup> )	1-800	do	+	+	+	+	+	+	50
( <sup>d</sup> )	1-1,600	do	+	+	+	+	+	+	50
1 to 200	1-1,600	do	+	+	+	+	+	+	50
Do	1-2,000	do	+	+	+	+	+	+	50
Do	1-2,400	do	+	+	+	+	+	+	50
1 to 400	1-1,600	do	+	+	+	+	+	+	50
Do	1-2,000	do	+	+	+	+	+	+	50
Do	1-2,400	do	+	+	+	+	+	+	50
1 to 800	1-800	do	+	+	+	+	+	+	50
Do	1-1,200	do	+	+	+	+	+	+	50
Do	1-1,600	do	+	+	+	+	+	+	50
Do	1-2,000	do	+	+	+	+	+	+	50
1 to 10	( <sup>b</sup> )	Skim milk, 50 per cent	+	+	+	+	+	+	40
( <sup>d</sup> )	1-100	do	+	+	+	+	+	+	40
1 to 50	1-50	do	+	+	+	+	+	+	40
Do	1-100	do	+	+	+	+	+	+	40
Do	1-200	do	+	+	+	+	+	+	40
Do	1-400	do	+	+	+	+	+	+	40
1 to 100	1-50	do	+	+	+	+	+	+	40
Do	1-100	do	+	+	+	+	+	+	40
Do	1-200	do	+	+	+	+	+	+	40
Do	1-400	do	+	+	+	+	+	+	40
1 to 40	( <sup>b</sup> )	Skim milk, 10 per cent	+	+	+	+	+	+	40
( <sup>d</sup> )	1-200	do	+	+	+	+	+	+	40
1 to 100	1-200	do	+	+	+	+	+	+	40
Do	1-400	do	+	+	+	+	+	+	40
Do	1-600	do	+	+	+	+	+	+	40
1 to 40	( <sup>b</sup> )	Skim milk, 50 per cent	+	+	+	+	+	+	50
( <sup>d</sup> )	1-200	do	+	+	+	+	+	+	50
1 to 100	1-100	do	+	+	+	+	+	+	50
Do	1-200	do	+	+	+	+	+	+	50
Do	1-400	do	+	+	+	+	+	+	50
Do	1-800	do	+	+	+	+	+	+	50

<sup>a</sup> + indicates growth; —, no growth.<sup>b</sup> No NaOH used.<sup>c</sup> Growth after 24 hours' exposure.<sup>d</sup> No Na<sub>2</sub>CO<sub>3</sub> used.<sup>e</sup> Growth after 1½ hours' exposure; no growth after 2 hours.<sup>f</sup> No growth after 2½ minutes' exposure.

In general, these results indicate that sodium carbonate itself has comparatively little germicidal efficiency at temperatures approximating 25° C. but that it is effective at higher temperatures, even in the presence of organic matter. The addition of sodium carbonate to solutions of sodium hydroxide greatly increases the efficiency of such solutions, and the efficiency of the mixtures is still further increased by heat. Tables 6 and 7 show the quantities of sodium carbonate in proportion to sodium hydroxide that are of value, and also that if more of the carbonate is used comparatively little is added to the germicidal efficiency of the solutions. The addition of sodium chloride to solutions of sodium hydroxide produces a comparatively small increase in efficiency at ordinary temperatures with practically no increase at higher temperatures.

Results of tests with mixtures containing sodium carbonate and various soaps, shown in Table 8, indicate that the carbonate considerably increases the germicidal efficiency of soap solutions.

TABLE 8.—Germicidal efficiency of mixtures containing sodium carbonate and soap against *Salmonella pullorum* <sup>a</sup>

Na <sub>2</sub> CO <sub>3</sub> dilution	Soap	Soap dilution	Growth <sup>b</sup> after indicated period of exposure (minutes)					
			5	10	15	20	25	30
(c).....	Coconut oil.....	1-20	—	—	—	—	—	—
(c).....	do.....	1-30	—	—	—	—	—	—
(c).....	do.....	1-40	+	+	+	+	+	+
(c).....	Castor oil.....	1-10	+	+	+	+	+	+
(c).....	Linseed oil.....	1-10	+	+	+	+	+	+
1 to 50.....	None.....	.....	+	+	+	+	+	+
1 to 100.....	do.....	.....	+	+	+	+	+	+
1 to 200.....	Castor oil.....	1-500	+	+	+	+	+	+
Do.....	do.....	1-1,000	+	+	+	+	+	+
Do.....	do.....	1-1,500	+	+	+	+	+	+
Do.....	do.....	1-1,200	+	+	+	+	+	+
Do.....	do.....	1-2,000	+	+	+	+	+	+
Do.....	Coconut oil.....	1-500	+	+	+	+	+	+
Do.....	do.....	1-1,000	+	+	+	+	+	+
Do.....	do.....	1-1,500	+	+	+	+	+	+
Do.....	do.....	1-2,000	+	+	+	+	+	+
Do.....	do.....	1-2,500	+	+	+	+	+	+
Do.....	Linseed oil.....	1-25	+	+	+	+	+	+
Do.....	do.....	1-50	+	+	+	+	+	+
Do.....	do.....	1-100	+	+	+	+	+	+
Do.....	do.....	1-200	+	+	+	+	+	+
Do.....	do.....	1-400	+	+	+	+	+	+
Do.....	do.....	1-800	+	+	+	+	+	+
Do.....	do.....	1-1,200	+	+	+	+	+	+
Do.....	do.....	1-1,600	+	+	+	+	+	+
Do.....	do.....	1-2,000	+	+	+	+	+	+
Do.....	do.....	1-2,400	+	+	+	+	+	+

<sup>a</sup> Experiments conducted at 25° C.

<sup>b</sup> + indicates growth; —, no growth.

<sup>c</sup> No Na<sub>2</sub>CO<sub>3</sub> used.

The results of experiments with trisodium phosphate and mixtures containing trisodium phosphate and sodium hydroxide are shown in Table 9, and results obtained with mixtures containing trisodium phosphate and soaps are shown in Table 10. These results indicate that trisodium phosphate has greater germicidal efficiency than sodium carbonate and that it is also more effective in increasing the germicidal efficiency of sodium hydroxide solutions and of soap solutions except in some instances when *Staphylococcus aureus* was used.

TABLE 9.—*Germicidal efficiency against Salmonella pullorum and Staphylococcus aureus of trisodium phosphate and its mixtures with sodium hydroxide without and with organic matter present*

Na <sub>3</sub> PO <sub>4</sub> dilution	NaOH dilution	Organic matter	Growth * after indicated period of exposure (minutes)						Medica- tion tem- perature	Test organism
			5	10	15	30	45	60		
1 to 200.....	( <sup>b</sup> )	None.....	—	—	—	—	—	—	( <sup>c</sup> )	S. pullorum.
1 to 400.....	( <sup>b</sup> )	do.....	+	+	+	—	—	—	( <sup>c</sup> )	Do.
1 to 600.....	( <sup>b</sup> )	do.....	+	+	+	+	+	+	( <sup>c</sup> )	Do.
( <sup>d</sup> ).....	1-1,500	do.....	+	+	+	+	+	+	( <sup>c</sup> )	Do.
( <sup>d</sup> ).....	1-2,000	do.....	+	+	+	+	+	+	( <sup>c</sup> )	Do.
1 to 600.....	1-2,000	do.....	+	+	—	—	—	—	( <sup>c</sup> )	Do.
Do.....	1-3,000	do.....	+	+	—	—	—	—	( <sup>c</sup> )	Do.
Do.....	1-4,000	do.....	+	+	+	—	—	—	( <sup>c</sup> )	Do.
1 to 50.....	( <sup>b</sup> )	do.....	+	+	+	+	+	+	( <sup>c</sup> )	Staph. aureus.
( <sup>d</sup> ).....	1-100	do.....	+	+	+	—	—	—	( <sup>c</sup> )	Do.
( <sup>d</sup> ).....	1-200	do.....	+	+	+	—	—	—	( <sup>c</sup> )	Do.
1 to 200.....	1-200	do.....	+	+	+	—	—	—	( <sup>c</sup> )	Do.
Do.....	1-400	do.....	+	+	+	+	+	—	( <sup>c</sup> )	Do.
1 to 50.....	( <sup>b</sup> )	do.....	+	+	—	—	—	—	40	Do.
1 to 100.....	( <sup>b</sup> )	do.....	+	+	—	—	—	—	40	Do.
( <sup>d</sup> ).....	1-400	do.....	+	+	+	—	—	—	40	Do.
1 to 200.....	1-400	do.....	+	+	—	—	—	—	40	Do.
Do.....	1-800	do.....	+	+	+	—	—	—	40	Do.
Do.....	1-1,200	do.....	+	+	+	—	—	—	40	Do.
Do.....	( <sup>b</sup> )	do.....	+	+	—	—	—	—	50	Do.
1 to 400.....	( <sup>b</sup> )	do.....	+	+	+	+	—	—	50	Do.
( <sup>d</sup> ).....	1-600	do.....	+	+	+	—	—	—	50	Do.
( <sup>d</sup> ).....	1-800	do.....	+	+	+	—	—	—	50	Do.
1 to 400.....	1-1,600	do.....	+	+	—	—	—	—	50	Do.
Do.....	1-2,000	do.....	+	+	—	—	—	—	50	Do.
1 to 800.....	1-800	do.....	+	+	—	—	—	—	50	Do.
Do.....	1-1,200	do.....	+	+	—	—	—	—	50	Do.
1 to 100.....	( <sup>b</sup> )	Skim milk, 50 per cent.....	+	+	—	—	—	—	( <sup>c</sup> )	S. pullorum.
1 to 200.....	( <sup>b</sup> )	do.....	+	+	+	+	+	+	( <sup>c</sup> )	Do.
( <sup>d</sup> ).....	1-400	do.....	+	+	+	—	—	—	( <sup>c</sup> )	Do.
( <sup>d</sup> ).....	1-500	do.....	+	+	+	—	—	—	( <sup>c</sup> )	Do.
1 to 200.....	1-800	do.....	+	+	—	—	—	—	( <sup>c</sup> )	Do.
Do.....	1-1,200	do.....	+	+	—	—	—	—	( <sup>c</sup> )	Do.
Do.....	1-1,600	do.....	+	+	+	—	—	—	( <sup>c</sup> )	Do.
1 to 25.....	( <sup>b</sup> )	do.....	+	+	+	—	—	—	40	Staph. aureus.
1 to 50.....	( <sup>b</sup> )	do.....	+	+	+	+	—	—	40	Do.
( <sup>d</sup> ).....	1-100	do.....	+	+	—	—	—	—	40	Do.
( <sup>d</sup> ).....	1-200	do.....	+	+	+	—	—	—	40	Do.
1 to 50.....	1-200	do.....	+	+	+	—	—	—	40	Do.
Do.....	1-400	do.....	+	+	+	—	—	—	40	Do.
1 to 100.....	1-200	do.....	+	+	+	—	—	—	40	Do.
Do.....	1-400	do.....	+	+	+	—	—	—	40	Do.
1 to 50.....	( <sup>b</sup> )	Skim milk, 10 per cent.....	+	+	—	—	—	—	40	Do.
1 to 100.....	( <sup>b</sup> )	do.....	+	+	—	—	—	—	40	Do.
( <sup>d</sup> ).....	1-200	do.....	+	+	+	—	—	—	40	Do.
( <sup>d</sup> ).....	1-400	do.....	+	+	+	—	—	—	40	Do.
1 to 50.....	1-800	do.....	+	+	—	—	—	—	40	Do.
Do.....	1-1,200	do.....	+	+	—	—	—	—	40	Do.
1 to 100.....	1-400	do.....	+	+	—	—	—	—	40	Do.
Do.....	1-800	do.....	+	+	—	—	—	—	40	Do.
1 to 50.....	( <sup>b</sup> )	Skim milk, 50 per cent.....	+	+	+	—	—	—	50	Do.
1 to 100.....	( <sup>b</sup> )	do.....	+	+	+	—	—	—	50	Do.
( <sup>d</sup> ).....	1-200	do.....	+	+	+	—	—	—	50	Do.
( <sup>d</sup> ).....	1-400	do.....	+	+	+	+	—	—	50	Do.
1 to 50.....	1-800	do.....	+	+	—	—	—	—	50	Do.
Do.....	1-1,200	do.....	+	+	—	—	—	—	50	Do.
1 to 100.....	1-400	do.....	+	+	—	—	—	—	50	Do.
Do.....	1-800	do.....	+	+	—	—	—	—	50	Do.

\* + indicates growth; —, no growth.

<sup>b</sup> No NaOH used.<sup>c</sup> Room temperatures between 22° and 32° C.<sup>d</sup> No Na<sub>3</sub>PO<sub>4</sub> used.

TABLE 10.—*Germicidal efficiency of mixtures containing trisodium phosphate and soap against Salmonella pullorum and Staphylococcus aureus*<sup>a</sup>

Na <sub>3</sub> PO <sub>4</sub> dilution	Soap	Soap dilution	Growth <sup>b</sup> after indicated period of exposure (minutes)						Test organism
			5	10	15	30	45	60	
1 to 600	Castor oil	1-1,000	+	—	—	—	—	—	<i>S. pullorum</i> .
Do	do	1-2,000	+	+	+	—	—	—	Do.
Do	do	1-3,000	+	+	+	+	—	—	Do.
Do	do	1-4,000	+	+	+	+	+	—	Do.
Do	Coconut oil	1-1,000	+	—	—	—	—	—	Do.
Do	do	1-2,000	+	+	+	—	—	—	Do.
Do	do	1-3,000	+	+	+	+	—	—	Do.
Do	do	1-4,000	+	+	+	+	+	—	Do.
(c)	do	1-5	+	+	+	+	+	+	<i>Staph. aureus</i> .
1 to 100	do	1-20	+	+	+	—	—	—	Do.
Do	do	1-40	+	+	+	—	—	—	Do.
Do	do	1-60	+	+	+	—	—	—	Do.
Do	do	1-80	+	+	+	—	—	—	Do.
Do	do	1-100	+	+	+	—	—	—	Do.
1 to 200	do	1-60	+	+	+	—	—	—	Do.
Do	do	1-80	+	+	+	—	—	—	Do.
Do	do	1-100	+	+	+	+	+	+	Do.

<sup>a</sup> Experiments conducted at room temperature (between 22° and 32° C.).<sup>b</sup> + indicates growth, —, no growth.<sup>c</sup> No Na<sub>3</sub>PO<sub>4</sub> used.

The results of experiments with sodium hydroxide and calcium hydroxide against *Staphylococcus aureus*, shown in Table 11, indicate that calcium hydroxide increases the germicidal efficiency of sodium hydroxide against that test organism.

TABLE 11.—*Germicidal efficiency of sodium hydroxide and calcium hydroxide against Staphylococcus aureus*<sup>a</sup>

Ca(OH) <sub>2</sub> dilution	NaOH dilution	Organic matter	Growth <sup>b</sup> after indicated period of exposure (minutes)								
			5	10	15	20	30	45	60	90	120
(c)	1 to 100	None	+	—	—	—	—	—	—	—	—
(c)	1 to 200	do	+	+	+	+	—	—	—	—	—
1 to 100	(d)	do	+	+	+	+	+	—	—	—	—
1 to 20	(d)	do	+	+	+	+	+	—	—	—	—
Do	1 to 100	do	+	+	—	—	—	—	—	—	—
Do	1 to 200	do	+	+	—	—	—	—	—	—	—
Do	1 to 400	do	+	+	+	—	—	—	—	—	—
(c)	1 to 50	Chicken feces	+	+	—	—	—	—	—	—	—
(c)	1 to 100	do	+	+	+	+	+	+	+	+	+
1 to 100	(d)	do	+	+	+	+	+	+	+	—	—
1 to 20	(d)	do	+	+	+	+	+	+	—	—	—
1 to 100	1 to 100	do	+	+	+	+	—	—	—	—	—
1 to 20	1 to 50	do	+	—	—	—	—	—	—	—	—
Do	1 to 100	do	+	+	+	—	—	—	—	—	—
Do	1 to 200	do	+	+	+	—	—	—	—	—	—
Do	1 to 400	do	+	+	+	—	—	—	—	—	—
Do	1 to 800	do	+	+	+	+	+	+	+	—	—

<sup>a</sup> Experiments conducted at room temperature (between 22° and 32° C.).<sup>b</sup> + indicates growth; —, no growth.<sup>c</sup> No Ca(OH)<sub>2</sub> used.<sup>d</sup> No NaOH used.<sup>e</sup> 1 gm. chicken feces to 5 c. c. disinfectant.

Table 12 presents the results of experiments with sodium hydroxide and with mixtures of sodium hydroxide and calcium hydroxide against spores of *Bacillus anthracis*. In experiment 1, one-half of a cubic centimeter of a spore suspension containing approximately 1,000,000 spores per cubic centimeter was added to 5 c. c. of disinfectant; whereas in experiment 2, 5 c. c. of disinfectant was added to a mixture containing 4 c. c. of defibrinated horse blood and 1 c. c. of the above-mentioned spore suspension. It is obvious, therefore, that in experiment 2 the initial concentrations of sodium hydroxide and calcium hydroxide were necessarily just twice as great as the concentrations shown in the table. Results obtained with mercuric chloride are shown only for comparison.

TABLE 12.—*Germicidal efficiency of sodium hydroxide and of sodium hydroxide with calcium hydroxide against spores of Bacillus anthracis*

EXPERIMENT 1<sup>a</sup>

Ca (OH) <sub>2</sub> NaOH		Growth <sup>b</sup> after indicated period of exposure (minutes)					
		15	30	45	60	90	120
Per cent	Per cent						
0	10	+	—	—	—	—	—
0	8	+	+	—	—	—	—
0	6	+	+	—	—	—	—
0	5	+	+	+	—	—	—
0	4	+	+	+	+	—	—
5	5	+	+	+	+	—	—
10	5	+	+	+	+	—	—
5	4	+	+	+	+	—	—
10	4	+	+	+	+	—	—

EXPERIMENT 2<sup>a</sup>

Blood	Ca (OH) <sub>2</sub>	NaOH	Growth <sup>b</sup> after indicated period of exposure (hours)					
			1	1½	2	3	4	5
Per cent	Per cent	Per cent						
40	0	5.0	+	—	—	—	—	—
40	0	2.5	+	+	+	—	—	—
40	2.5	2.5	+	+	+	+	—	—
40	5.0	2.5	+	+	+	+	—	—
40	( <sup>c</sup> )	( <sup>d</sup> )	+	+	+	+	+	+

<sup>a</sup> Experiment conducted at room temperature, approximately 32° C.

<sup>b</sup> + indicates growth, —, no growth.

<sup>c</sup> HgCl<sub>2</sub> used.

<sup>d</sup> Dilution of 1-2,000.

It is well known that sodium hydroxide in solution when exposed to the air is converted to sodium carbonate. In view of this fact, experiments were conducted to ascertain the possible effect of such conversion upon germicidal efficiency. The results of the bacteriological tests are shown in Table 13, and the results of the chemical tests in Table 14.

TABLE 13.—*Germicidal efficiency of sodium hydroxide and of sodium hydroxide and calcium hydroxide against various organisms in open and closed containers*

EXPERIMENT 1<sup>a</sup>

Organic matter (blood)	Ca (OH) <sub>2</sub>	NaOH	Growth <sup>b</sup> after indicated period of exposure (hours)						Container	Test organism
			1	1½	2	3	4	5		
<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>								
40	0	5.0	+	—	—	—	—	—	Closed.....	Spores of Bacillus anthracis.
40	0	5.0	+	—	—	—	—	—	Open.....	Do.
40	0	2.5	+	+	+	—	—	—	Closed.....	Do.
40	0	2.5	+	+	+	—	—	—	Open.....	Do.
40	2.5	2.5	+	+	+	+	—	—	Closed.....	Do.
40	2.5	2.5	+	+	+	—	—	—	Open.....	Do.

EXPERIMENT 2<sup>a</sup>

Organic matter (skim milk)	Ca (OH) <sub>2</sub>	NaOH dilution	Growth <sup>b</sup> after indicated period of exposure (minutes)						Container	Test organism
			5	10	15	20	25	30		
<i>Per cent</i>	<i>Per cent</i>									
0	0	1-150	+	+	—	—	—	—	Closed.....	Staphylococcus aureus
0	0	1-150	+	+	—	—	—	—	Open.....	Do.
50	0	1-120	+	+	+	+	—	—	Closed.....	Do.
50	0	1-120	+	+	+	—	—	—	Open.....	Do.
0	0	1-1,200	+	—	—	—	—	—	do.....	Salmonella pullorum.
0	0	1-1,500	+	—	—	—	—	—	Closed.....	Do.
0	0	1-1,500	+	+	+	+	+	+	Open.....	Do.
50	0	1-400	—	—	—	—	—	—	Closed.....	Do.
50	0	1-400	—	—	—	—	—	—	Open.....	Do.
50	0	1-500	+	+	+	—	—	—	Closed.....	Do.
50	0	1-500	+	+	+	+	+	+	Open.....	Do.

<sup>a</sup> Experiment conducted at room temperature, approximately 32° C.

<sup>b</sup> + indicates growth; —, no growth.

TABLE 14.—*Conversion of sodium hydroxide to sodium carbonate on exposure to air*

EXPERIMENT 1

Initial NaOH present	Period of exposure	Final NaOH present	Na <sub>2</sub> CO <sub>3</sub> formed	Unchanged NaOH
<i>Per cent</i>	<i>Minutes</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1	15	0.67	0.60	60
1	30	.33	1.24	26
2	15	1.56	.86	71
2	30	1.44	1.21	61

EXPERIMENT 2

1	1	-----	-----	80-90
1	5	-----	-----	40
1	10	-----	-----	10
2	1	-----	-----	90-95
2	5	-----	-----	43
2	15	-----	-----	0

EXPERIMENT 3

	<i>Hours</i>			
1	18	-----	-----	64
1	24	-----	-----	53
2	18	-----	-----	73
2	24	-----	-----	64
2+10 per cent Ca (OH) <sub>2</sub>	48	-----	-----	100



In experiment 2 (Table 13) the open containers were uncovered Petri dishes, whereas in experiment 1 they were sterile glass plates on which the material to be disinfected was spread out. In both experiments the "closed" containers were test tubes closed with cotton plugs.

Other experiments were conducted with sterile strips of filter paper as test objects. These strips were soaked in a culture of *Staphylococcus aureus* and then transferred to open Petri dishes and allowed to dry for about 10 minutes. A sodium-hydroxide solution was then poured into the dishes, and the filter-paper strips were thoroughly wet with the solution. Half of the strips were spread out in dry, open Petri dishes, whereas the others remained in contact with the sodium-hydroxide solution in closed dishes. The results obtained were similar to those shown for *S. aureus* in experiment 2 of Table 13.

In experiment 1 of Table 14, weighed filter papers were soaked in sodium-hydroxide solution, weighed again to determine the amount of solution taken up, and then exposed to the air in shallow dishes. At the end of each exposure period one of the filter papers was weighed again, and then transferred to a beaker containing distilled water, and thoroughly washed. The resulting solution was immediately titrated with suitable indicators to determine the amounts of sodium hydroxide and sodium carbonate present. The percentage figures in the fifth column were corrected to allow for evaporation. The differences between these figures and those shown in the third column indicate the amount of relative increase in concentration of sodium hydroxide due to evaporation.

In experiment 2 of Table 14, solutions of sodium hydroxide were sprayed in a fine mist across 2 feet of space upon filter papers fastened to the wall. At the end of the exposure periods the papers were washed off in distilled water, and the resulting solutions were titrated for sodium hydroxide and sodium carbonate.

In experiment 3 of Table 14, solutions of sodium hydroxide, with and without lime, were exposed to the air in shallow dishes. The results indicate that the lime acted to maintain the concentration of the sodium hydroxide. In tightly covered containers there was no appreciable change of hydroxide to carbonate even after a considerable length of time.

#### DISCUSSION

The results shown in the preceding tables indicate that sodium hydroxide, by itself or in mixtures with sodium carbonate, trisodium phosphate or calcium hydroxide, is an efficient disinfectant for use against many of the spore-bearing bacteria and also against the spores of *Bacillus anthracis*. The work of the American commission to study foot-and-mouth disease<sup>4</sup> has shown that sodium hydroxide is very effective against the virus of that disease. According to Helm and Wedemann,<sup>5</sup> the addition of lime to sodium-hydroxide solutions increases their efficiency against the virus of foot-and-mouth disease. On the other hand, the work of Petroff<sup>6</sup> and of Corper and

<sup>4</sup> OLITSKY, P. K., TRAUM, J., and SCHOENING, H. W. REPORT OF THE FOOT-AND-MOUTH-DISEASE COMMISSION OF THE UNITED STATES DEPARTMENT OF AGRICULTURE. U. S. Dept. Agr. Tech. Bul. 76, 172 p., illus. 1928.

<sup>5</sup> HELM, R. and WEDEMANN, W. VERSUCHE MIT VERSCHIEDENEN DISINFEKTIONSMITTELN ZUR ABTÖTUNG DES VIRUS DER MAUL-UND-KLAUSENSEUCHE. Arb. Reichsgesundheitsamt. [Germany] 61: 569-594. 1930.

<sup>6</sup> PETROFF, S. A. A NEW AND RAPID METHOD FOR THE ISOLATION AND CULTIVATION OF TUBERCLE BACILLI DIRECTLY FROM SPUTUM AND FECES. Jour. Expt. Med. 21: 38-42. 1915.

Uyei<sup>7</sup> indicates that sodium hydroxide has practically no germicidal efficiency against the tubercle bacillus. During the course of the present work the writers tested mixtures containing 2 per cent of sodium hydroxide and 10 per cent of lime against the tubercle bacillus and found them to be ineffective after two hours' exposure.

For purposes of general disinfection it is suggested that sodium hydroxide should be used in 2 per cent solution with or without lime. When circumstances permit its use, the solution containing lime will no doubt be preferred, since the lime not only serves to indicate where the solution has been applied but also increases germicidal efficiency, and tends to maintain the strength of the sodium hydroxide. When spore-bearing organisms are to be destroyed, it is suggested that sodium hydroxide, with or without lime, should be used with initial concentrations of from 5 to 10 per cent.

Sodium hydroxide is available commercially as caustic soda or lye in a very pure state. Since caustic soda which assays more than 90 per cent sodium hydroxide is readily obtainable, it would seem that a good grade of the commercial product might be used for practical disinfecting operations.

Although caustic soda itself and concentrated solutions of it are corrosive, this injurious effect is greatly diminished by dilution. The dilute solutions here suggested for general disinfection may be used with safety, provided a reasonable degree of care is exercised. Such solutions have little or no harmful effect upon rubber, bare wood, or cotton; but repeated treatment is harmful to wool or silk. Leather, especially when oiled, and many kinds of painted surfaces withstand some exposure to these solutions, but it is better to avoid unnecessary exposure. The solutions may be kept in vessels of wood, earthenware, enameled ware, or any of the common metals with the exception of aluminum. Containers should be tightly covered to prevent conversion of hydroxide to carbonate by exposure to air. In handling the stronger solutions suggested for use against spore-bearing organisms, special care should be exercised.

For cleansing and disinfecting it is suggested that sodium carbonate be used in a 2 per cent solution. This may be obtained by using commercial soda at the rate of 6 parts of soda crystals, or 2 parts of soda ash, in 100 parts of water; or trisodium phosphate may be used in a 1 per cent solution, which may be obtained by using the commercial tribasic phosphate of soda in the proportion of 2.5 parts in 100 parts of water. If these solutions are to be used at ordinary temperatures, it is advisable to add 0.5 per cent of sodium hydroxide. If the solutions are to be used hot, the addition of sodium hydroxide will not be necessary.

#### SUMMARY

Bacteriological tests were made with sodium hydroxide, sodium carbonate, trisodium phosphate, mixtures containing sodium carbonate or trisodium phosphate with sodium hydroxide or with soaps, and mixtures containing sodium hydroxide and calcium hydroxide. The test organisms used were *Eberthella typhi*, *Staphylococcus aureus*, *Salmonella pullorum*, *Salmonella gallinarum*, *Pasteurella avicida*, two strains of hemolytic streptococci, and spores of *Bacillus anthracis*.

<sup>7</sup> CORPER, H. J. and UYEI, N. THE ISOLATION OF TUBERCLE BACILLI FROM CONTAMINATED TUBERCULOUS MATERIALS. *Tubercle* 9: 115-130. 1927.

Sodium hydroxide, alone or in mixtures with sodium carbonate, trisodium phosphate, or calcium hydroxide, was found to have relatively high germicidal efficiency against all these organisms, even in the presence of organic matter in the form of skim milk, chicken feces, or defibrinated horse blood. During the course of the work it was found that solutions containing 2 per cent sodium hydroxide and 10 per cent calcium hydroxide failed to kill *Mycobacterium tuberculosis* without organic matter after two hours' exposure.

Sodium carbonate, trisodium phosphate, and calcium hydroxide each had comparatively little germicidal efficiency at ordinary temperatures, but when added to solutions of sodium hydroxide they increased the germicidal efficiency of these solutions. The germicidal efficiency of solutions of sodium carbonate or trisodium phosphate or of mixtures containing either of these compounds with sodium hydroxide was greatly increased by heat. The addition of sodium carbonate or trisodium phosphate to soap solutions increased their germicidal efficiency.

# A METHOD FOR THE PREPARATION AND ANALYSIS OF REPRESENTATIVE SAMPLES FROM THE BOVINE SKELETAL STRUCTURE<sup>1</sup>

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## INTRODUCTION

In the past, studies of the composition of the skeletal structure of animals have been limited to rather gross analyses; moisture, fat, and ash being the constituents usually determined. Recently, however, methods have been devised for more detailed studies, so that the constituents of the mineral matter can also be determined. Information so gained is necessary for an understanding of the complete effects of various deficiencies in nutrition.

When animals that had been used in mineral nutrition projects at the Minnesota station were slaughtered an opportunity was afforded to study the effect of age and nutrition on the skeletal structure of cattle. The old method of grinding an entire half skeleton requires a large amount of labor. Other methods lack experimental support. The method to be described was developed to avoid this labor, and yet to furnish a sample that would adequately represent the skeleton. Preliminary studies indicated that comparative results could be secured, and the data obtained support this conclusion.

The use of the femur, humerus, and ribs in small-animal experimentation prompted their selection to represent the entire skeleton of cattle in this study. These bones represent both endochondral and intramembranous ossification, and hence should be affected by changes in nutrition proportionately with the other bones. However, there is as yet no direct evidence on this point.

A considerable amount of labor is required to reduce a whole femur or humerus from a large cow into material suitable for analysis. In order to eliminate as much of this labor as possible and at the same time to reduce the size of the sample, various fractions of these bones were analyzed, and the results of the analyses were compared with those for the whole bone. It was concluded from this work that a longitudinal section could be sawed from either the femur or the humerus which would closely approximate the whole bone in composition. The method employed will first be described, and then the data in support of it.

## THE METHOD

The femur, humerus, sixth and eleventh ribs from the right side of the bovine carcass were removed as soon as possible after the animal was slaughtered. These bones with the adhering flesh were

<sup>1</sup> Received for publication Aug. 28, 1930; issued January, 1931. This, the first of two articles relating to the influence of age and nutrition on the major mineral constituents of the skeletal structure of cattle, was presented by the senior author to the faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy, June, 1929. Paper No. 949, Journal Series, Minnesota Agricultural Experiment Station.

<sup>2</sup> Resigned June 30, 1929.

frozen immediately. When this was done the flesh was scraped from the bone and longitudinal sections of the femur and humerus were sawed. These sections and the ribs were then broken in an iron mortar and weighed into tared aluminum dishes. The type of saw used and the character of the bone samples are shown in Figure 1. The longitudinal section shown is of uniform thickness when prepared with the modified hack saw. It is obtained most easily from the frozen bone. This section more nearly represents proportionately the

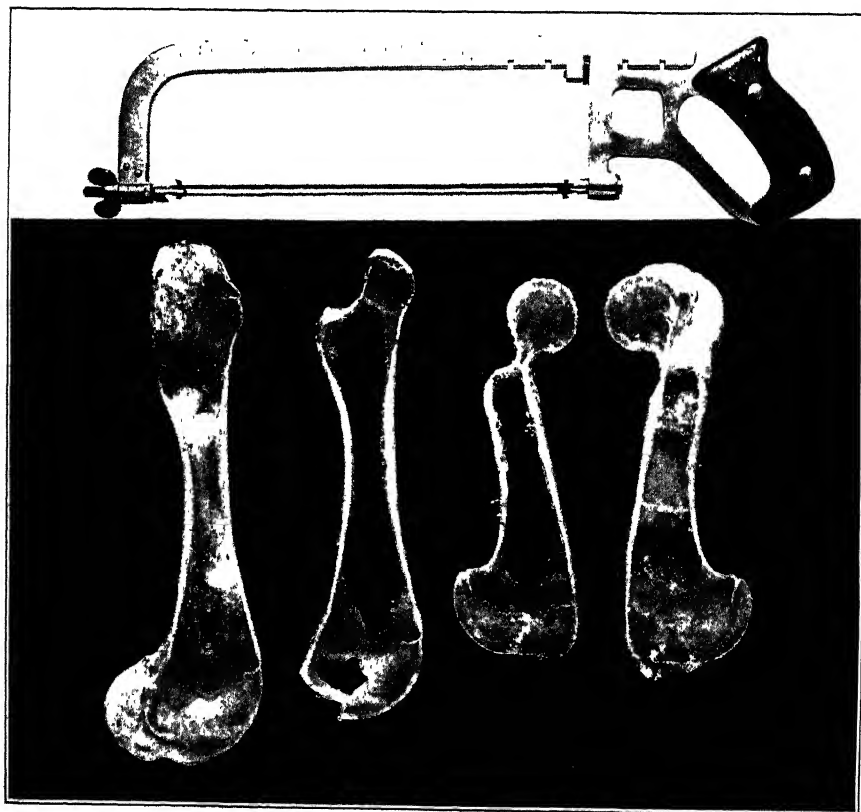


FIGURE 1.—Double-bitted hack saw used to cut longitudinal sections of bone for samples as shown

different parts of the bone than the cross section usually made. No other equally representative sample of a large bone can be secured so easily or prepared for analysis with less labor.

All samples were dried to constant weight at 100° C. When dried they were transferred to extraction thimbles, placed in a Lloyd drug extractor, and extracted with hot 95 per cent alcohol followed by ether until they were extract-free. The percentage of alcohol-ether extract was calculated from the difference between the dry and dry-extracted weights. The sample was then reduced in an iron mortar until it would pass a 20-mesh sieve. Large samples were ground in a Hobart mill. Ash determinations were made on gram aliquots of this bone powder.

The mineral constituents of the dry extracted bone were determined by the method of Kramer and Howland<sup>3</sup> for unashed bone, except for two modifications. One modification was the precipitation, washing, and titration of the calcium in 75 c. c. pointed centrifuge tubes as for blood calcium,<sup>4</sup> but using the quantities of sample and reagents recommended by Kramer and Howland. The other was the determination of the carbonate by absorption in standard alkali, using a set-up very similar to that employed in the Geissler method. (Fig. 2.)

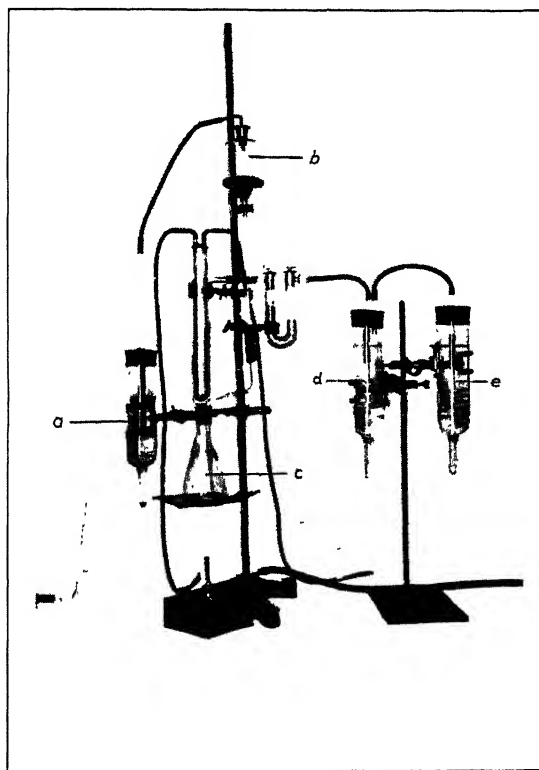


FIGURE 2.—Apparatus for the determination of carbonate by  $\text{CO}_2$  absorption in standard alkali: *a*, Absorption tube containing 40 per cent KOH solution; *b*, separatory funnel for adding HCl for liberation of  $\text{CO}_2$ ; *c*, flask containing sample of bone powder, *d-e*, tubes containing standard alkali for absorption of  $\text{CO}_2$

A 1.25 gm. sample of bone powder was weighed into the flask *c*, and 25 c. c. of 1 N hydrochloric acid was added through the separatory funnel *b* for the liberation of the carbon dioxide. Carbon dioxide-free air was furnished by aspiration through the absorption tube *a* containing 40 per cent potassium hydroxide solution. Aspiration and heating were continued for 30 minutes at the rate of about two bubbles per second. The reflux condenser and the U-tube con-

<sup>3</sup> KRAMER, B., and HOWLAND, J. THE QUANTITATIVE ESTIMATION OF CALCIUM, MAGNESIUM, PHOSPHATE, AND CARBONATE IN BONE. *Jour. Biol. Chem.* 63: 711-719. 1926.

<sup>4</sup> KRAMER, B., and TISDALL, F. F. A SIMPLE TECHNIQUE FOR THE DETERMINATION OF CALCIUM AND MANGANESE IN SMALL AMOUNTS OF SERUM. *Jour. Biol. Chem.* 47: 475-481. 1921.

taining sulphuric acid (specific gravity 1.4) saturated with silver sulphate prevented any hydrochloric acid from contaminating the standard alkali in the tubes *d* and *e*. A 50 c. c. portion of alkali (1 c. c. equal 1 mgm.  $\text{CO}_2$ ) was used in each of these tubes.

After the absorption of the carbon dioxide was completed, 5 c. c. of 10 per cent barium chloride was added to each tube and the excess alkali titrated with standard acid, phenolphthalein being used as the indicator.

After the carbonate determination the sample was filtered into a volumetric flask, and aliquots were taken for the calcium, magnesium, and phosphorus determinations. The aliquots taken were equivalent in amount to those used in the Kramer and Howland<sup>5</sup> method. From the data obtained the ratios of residual calcium to residual phosphorus and of calcium phosphate to calcium carbonate were calculated according to the Kramer and Howland method.

### EXPERIMENTAL DATA

The analyses of the various fractions of a femur and of two longitudinal sections of a humerus as well as the whole bones of E-73 are given in Table 1. With the exception of fraction 7 none of the other fractions of the femur compared so closely in all respects with the whole bone as did the longitudinal section. Fraction 7 was not chosen because of the obvious difficulty of duplicating it in other bones and also because its mechanical reduction for analysis is just as difficult as in the case of the entire bone. The two sections of the humerus agree sufficiently well to show that a uniform section may be taken. That the percentage of ash in the dry extracted section is higher than that for the whole bone is due, probably, to a slightly higher proportion of the shaft being included in the section. The section is of uniform thickness, while the whole bone is thicker at the extremities. However, it may be supposed that this disproportionality would occur in all samples thus taken, so that the samples would still be comparable one with another.

TABLE 1.—Comparison of percentage composition of various fractions<sup>a</sup> of femur and humerus, and whole bone

Basis of calculation	Moisture (green) bone	Alcohol ether extract		Ash		
		Green bone	Dry bone	Green bone	Dry bone	Extract bone
Femur E-73:	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Longitudinal section.....	12.23	34.53	39.34	35.24	40.15	66.19
Fraction 1.....	19.08	36.37	44.95	27.26	33.68	61.19
Fraction 2.....	13.72	26.47	30.68	38.01	44.07	63.57
Fraction 3.....	13.39	33.47	38.65	32.19	37.17	60.59
Fraction 4.....	13.43	21.50	24.83	42.17	48.71	64.80
Fraction 5.....	12.18	35.01	39.86	34.50	39.29	65.33
Fraction 6.....	12.17	19.63	22.36	45.02	51.26	66.03
Fraction 7.....	12.83	30.85	35.16	36.64	42.03	64.83
Fraction 8.....	12.29	35.44	40.41	34.26	39.06	65.55
Whole bone.....	14.04	31.23	36.33	34.88	40.58	63.73
Humerus E-73:						
First longitudinal section.....	10.80	32.02	35.91	37.00	41.49	64.73
Second longitudinal section.....	11.65	31.45	35.59	36.81	41.67	64.70
Whole bone.....	11.09	33.67	37.87	34.76	39.09	62.84

<sup>a</sup> After the section was sawed, the half bone was divided again both longitudinally and crosswise through the shaft, each half yielding four fractions.

<sup>5</sup> KRAMER, B., and HOWLAND, J. Op. cit.

Since the analyses of these sections of the femur and humerus of E-73 gave results closely comparable to those for the whole bones, the work was continued, and the method outlined above was used on all available samples. Most attention was paid to the calcium phosphate/calcium carbonate ratio, as one of the objectives of the research was to determine the effect of different planes of calcium and phosphorus intake on the composition of the mineral matter in the skeleton.

The calcium phosphate/calcium carbonate ratios for the femur, humerus, and combined sample of the sixth and eleventh ribs for 16 animals considered normal, are given in Table 2.

TABLE 2.—Calcium phosphate/calcium carbonate ratios for the femur, humerus, and sixth and eleventh ribs of 16 normal animals

Animal No.	Age	Calcium phosphate/calcium carbonate ratio in—			
		Femur	Humerus	Ribs	Average ratio
	<i>Months</i>				
E-106.....	6	7.53	7.58	7.11	7.41
E-114.....	6	7.34	6.88	7.09	7.10
B-163.....	6	6.57	7.46	7.25	7.09
E-79.....	12	7.38	7.04	7.39	7.27
E-55.....	18	6.47	7.12	7.17	6.92
E-85.....	18	6.99	7.30	7.14	7.14
E-88.....	18	7.35	6.94	7.32	7.20
E-86.....	21	7.54	7.33	7.24	7.37
(a).....	( <sup>a</sup> )	7.87	7.72	7.97	7.85
E-78.....	24	7.38	7.46	7.58	7.47
E-98.....	24	6.75	6.70	6.52	6.66
E-23.....	( <sup>b</sup> )	6.91	6.69	6.68	6.76
310.....	( <sup>b</sup> )	6.70	6.07	6.78	6.50
101.....	( <sup>b</sup> )	6.88	6.47	6.42	6.59
19.....	( <sup>b</sup> )	6.56	6.40	6.28	6.41
F-62.....	( <sup>b</sup> )	6.44	6.96	6.91	6.77

<sup>a</sup> Jersey bull.

<sup>b</sup> Mature.

Correlations were calculated with the following results:

$$\begin{aligned}
 r_{\text{femur-humerus}} &= +0.53136 \pm 0.11736 \quad (N=16) \\
 r_{\text{femur-ribs}} &= +0.66739 \pm 0.09352 \quad (N=16) \\
 r_{\text{humerus-ribs}} &= +0.81416 \pm 0.05685 \quad (N=16) \\
 r_{\text{average-ribs}} &= +0.83353 \pm 0.05454 \quad (N=16)
 \end{aligned}$$

These correlations show that the calcium phosphate/calcium carbonate ratio of the humerus samples agrees very closely with that of the ribs, and that the ratio for the ribs is very close to the average ratio for the three samples. The ribs may thus be considered as the most reliable single sample.

It can not be concluded from these correlations, however, that the samples from the ribs give as accurate values as the means of the three samples. This is due to the unreliability of the last correlation coefficient caused by one variable being independent and the other dependent. Harris <sup>6</sup> has pointed out that a more significant value, the rate of variation of the dependent variable in relation to the independent variable, can be secured by a correlation of the independent variable with the deviation of the dependent variable from its most probable value. Applying Harris's <sup>7</sup> formula with the value

<sup>6</sup> HARRIS, J. A. THE CORRELATION BETWEEN A VARIABLE AND THE DEVIATION OF A DEPENDENT VARIABLE FROM ITS PROBABLE VALUE. *Biometrika* 6: 438-443. 1909.

<sup>7</sup> HARRIS, J. A. *Op. cit.*



for the ribs as the dependent variable, the value  $-0.13913 \pm 0.16536$  was obtained. This indicates that the ribs are a representative sample because the ratio for the ribs varies uniformly with the average for the three samples.

The availability of some cross sections of shaft made it desirable to determine whether the ratio obtained from the analysis of such a section would be comparable to that obtained from a longitudinal section as was adopted for this study. Table 3 gives a comparison of longitudinal and cross sections from the femurs and humeri of four animals.

TABLE 3.—Comparison of calcium phosphate/calcium carbonate and calcium/phosphorus ratios of longitudinal and cross sections of the same bone of animals on normal and low calcium rations

ANIMALS ON NORMAL CALCIUM RATIIONS						
Animal No.	Age	Bone	Ratios in—			
			Longitudinal sections		Cross section	
			$\frac{\text{Ca}_3(\text{PO}_4)_2}{\text{CaCO}_3}$	$\frac{^a \text{Ca}}{\text{P}}$	$\frac{\text{Ca}_3(\text{PO}_4)_2}{\text{CaCO}_3}$	$\frac{^a \text{Ca}}{\text{P}}$
E-106.....	Months 6	Femur.....	7.53	1.88	6.74	1.87
		Humerus.....	7.58	1.87	6.71	1.87
E-114.....	6	Femur.....	7.34	1.90	6.90	1.89
		Humerus.....	6.88	1.90	6.69	1.85
ANIMALS ON A LOW CALCIUM RATION						
E-109.....	12	Femur.....	6.95	1.92	7.00	1.86
		Humerus.....	6.89	1.87	6.57	1.87
E-110.....	12	Femur.....	7.41	1.90	7.08	1.88
		Humerus.....	7.11	1.90	7.04	1.88

<sup>a</sup> Residual.

The  $\frac{\text{Ca}_3(\text{PO}_4)_2}{\text{CaCO}_3}$  ratios for the samples from the animals on the low calcium rations agree fairly well. There is no such agreement for those from the animals on normal rations. The peculiarity of the low calcium ration apparently was to produce a mineral of more nearly the same composition as that already present in the shaft. Shear and Kramer<sup>8</sup> found differences in the composition of new and old calcification, and similar differences may account for the variations observed here. The necessity for a sample including all parts of the bone is evident.

When the analyses were completed, correlation coefficients were calculated as shown in Table 4. All animals from which the three samples had been taken are included. Fifteen animals had been on normal rations and 12 on abnormal mineral rations. These latter 12 are included in the total of 27. The lowest coefficient,  $+0.3919 \pm 0.1474$ , was between the magnesium values for the humeri and ribs. It is a little less than three times the probable error. Almost all the coefficients are greater than  $+0.7$  and the highest,  $+0.934933 \pm 0.016343$ , is unusually high for biological material.

<sup>8</sup> SHEAR, M. J., and KRAMER, B. COMPOSITION OF BONE.—III. PHYSICO-CHEMICAL MECHANISM. Jour. Biol. Chem. 79: 125-145. 1928.

On the assumption that there should be a high correlation between the quantity of any constituent in the various parts of the skeleton, these correlation coefficients indicate success in the selection of representative samples. The conclusion seems justified that longitudinal sections of the femur and humerus, and the sixth and eleventh ribs do adequately represent the bovine skeletal structure.

## SUMMARY

A method for the preparation and analysis of samples from the bovine skeleton has been described.

Data are presented to show that longitudinal sections of the femur and humerus and the sixth and eleventh ribs constitute representative samples of the bovine skeletal structure.

TABLE 4.—*Correlation coefficients calculated between the several analyses*<sup>a</sup>

CALCIUM					
Number of animals considered	r <sub>F-R</sub> <sup>b</sup>	r <sub>II-R</sub> <sup>b</sup>	r <sub>FII-R</sub> <sup>b</sup>	r <sub>HR-F</sub> <sup>b</sup>	r <sub>FR-H</sub> <sup>b</sup>
15	+0.6173±0.1093	+0.7332±0.0805			
27	+ .7686± .0700	+ .7143± .0636	+0.7267±0.0612	+0.8412±0.0422	+0.8490±0.0362
MAGNESIUM					
15	+ .5059±.1296	+ .3919± .1476			
27	+ .7906± .0487	+ .7361± .0595	+ .7817±.0505	+ .9082±.0227	+ .8592±.0340
PHOSPHORUS					
15	+ .5715±.1173	+ .7284±.0817			
27	+ .7210±.0623	+ .6314±.0780	+ .7217±.0622	+ .8344±.0394	+ .7740±.0520
CARBON DIOXIDE					
15	+ .7416± .0784	+ .7517±.0757			
27	+ .8799±.0293	+ .8866±.0278	+ .9039±.0237	+ .9228±.0193	+ .9294±.0187
CALCIUM PHOSPHATE/CALCIUM CARBONATE					
15	+ .6853±.0924	+ .8150±.0584	+ .8475±.0491	+ .6634±.0975	+ .7619±.0730
27	+ .8548±.0349	+ .9237±.0190	+ .9349±.0163	+ .8691±.0362	+ .9012±.0243
15	r <sub>FHR-R</sub> +0.9352±0.0218				

<sup>a</sup> Credit is due Rachel Rude, station assistant, for the calculation of the correlation coefficients presented in this table.

<sup>b</sup> F=femur, H=humerus, R=ribs, FII=average of femur and humerus, etc.



# EFFECT OF AGE AND NUTRITION ON THE CALCIUM PHOSPHATE/CALCIUM CARBONATE RATION IN THE BONES OF CATTLE<sup>1</sup>

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## INTRODUCTION

A knowledge of the changes induced in the skeletal structure of cattle by different planes of mineral nutrition should aid in determining the optimum mineral intake for the development of the skeleton and its maintenance in normal condition. The proportions as well as the total quantity of the mineral constituents may be altered through nutrition. Only in recent years have methods been devised for the study of these changes. Kramer and Howland (7)<sup>3</sup> provided such a method, and its adaptation for the analysis of the skeletons of cattle has been described in a previous paper (11).

This method of analysis permits the determination of calcium, magnesium, phosphorus, and carbonate on unashed bone. Since these comprise the constituents of the mineral matter, except for minute amounts of some other elements, it is possible to make deductions concerning some of the most probable salts and their proportions. By assuming that the carbonate is all present as calcium carbonate and the magnesium as trimagnesium phosphate, it was found that the remaining calcium and phosphorus were generally in nearly the same proportion as for tricalcium phosphate. Using this result in support of the assumption that the calcium phosphate is tricalcium phosphate, the ratio of tricalcium phosphate to calcium carbonate may be calculated.

In spite of the fact that only small variations may be secured in the Ca/P ration of bone ash (4, 5, 9, 10, 12), Howland, Marriott, and Kramer (6) and Kramer and Shear (8) found significant variations in the composition of calcification by analyzing unashed samples of bone. They reported the calcium phosphate/calcium carbonate ratio for the bones of normal rats to be 10.8 and 10.6, and for a rachitic rat 6.7. A similar reduction of the ratio was observed in rachitic human bones. A constant ratio would not be expected. The concentration of the ions in the body fluids surrounding the ossifying tissue is not constant and this should be reflected in the resulting ossification.

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<sup>1</sup> Received for publication Aug. 28, 1930; issued January, 1931. This, the second of two articles relating to the influence of age and nutrition on the major mineral constituents of the skeletal structure of cattle, was presented by the first-named author to the faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy, June, 1929. Paper No. 950, Journal Series, Minnesota Agricultural Experiment Station.

<sup>2</sup> Resigned June 30, 1929.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 121.

## EXPERIMENTAL DATA

So far as the writers are aware, no analyses of unashed bones of cattle have ever been reported. It was believed that such a study performed on the skeletons of cattle reared or maintained on rations of known calcium and phosphorus content would aid in the elucidation of the requirement for these elements. Normal mature cattle were taken from the University Farm herd where there has never been any evidence of mineral deficiency. Mature cattle that had received a phosphorus-deficient ration were available from the experiments of Eckles, Becker, and Palmer (3). Other animals used in the same project had received a phosphorus-deficient ration which was later supplemented with sodium or calcium phosphate. The younger animals were all from a project on the mineral requirements for growth. These latter animals were grouped according to the amount of calcium and phosphorus received.

Whether the supply of calcium and phosphorus was considered as low, medium, or high, was based on the computation made by Armsby (1) from the analyses of Lawes and Gilbert showing that the average daily storage is 3.2 gm. of calcium and 1.7 gm. of phosphorus per hundred pounds live weight. An animal receiving a moderate excess over these amounts was considered to be receiving a normal mineral ration. An average ration of mixed hay and grain supplies a slight excess of calcium and a liberal amount of phosphorus. Lack of data on the percentage assimilation of calcium and phosphorus by growing cattle precludes a definite statement as to the absolute adequacy of such a ration.

Natural feeds were used in all the rations and were assumed to supply sufficient vitamin D. Regardless of the quantitative intake of this vitamin, it was sufficiently uniform to prevent its being the determining factor in the composition of the mineral of the skeletal structure.

The ratio of residual calcium to residual phosphorus is not shown in this paper because in all cases it closely approximated the value for tricalcium phosphate, and so was not of additional significance.

Twenty animals were included in the normal group. The composition of the skeletons of the younger animals varied. Lack of absolute uniformity in the rations, individuality of the animals, and differences in their early history are probable causes of this variation. The average amount of calcium and phosphorus supplied was more than enough to care for the usual storage. All animals were normal in size and appearance.

Average ratios of the calcium phosphate to calcium carbonate obtained, as shown in Table 1, were: 7.32 for those slaughtered at 6, 9, and 12 months, 7.11 for those at 18 and 24 months, and 6.57 for mature cows. The decrease in the second year is very slight, but its occurrence is substantiated by the further decrease in mature cows—probably due to a more uniform mineral intake. These results for normal cattle serve as a standard of comparison for the cattle on abnormal mineral rations.

The cows on a known phosphorus-deficient ration received prairie hay grown in the phosphorus-deficient area in western Minnesota, ground oats, common salt, and water. The ratios for five such animals are presented in Table 2, the average for the low four being

5.30. E-37 was omitted from this average because she was not in milk, and no longer showed symptoms of mineral deficiency at the time of slaughter. A very liberal proportion of ground oats in her ration apparently had supplied her phosphorus requirement.

TABLE 1.—*Effect of age on the calcium phosphate/calcium carbonate ratio in the skeletons of dairy cattle on normal rations*

Animal No.	Age	$\frac{\text{Ca}_3(\text{PO}_4)_2}{\text{CaCO}_3}$	Standard deviation
	<i>Months</i>		
E-106.....	6	7.41	
E-114.....	6	7.10	
B-163.....	6	7.09	
E-102.....	9	7.73	
E-79.....	12	7.27	
Average.....		7.32	±0.237
E-55.....	18	6.92	
E-85.....	18	7.14	
E-88.....	18	7.20	
WVa-3 <sup>a</sup> .....	18	6.78	
WVa-6.....	18	7.23	
F-86.....	24	7.37	
E-78.....	24	7.47	
E-98.....	24	6.66	
WVa-4.....	24	7.02	
WVa-5.....	24	7.30	
Average.....		7.11	±.236
E-25.....	(b)	6.59	
E-23.....	(b)	6.76	
310.....	(b)	6.50	
101.....	(b)	6.59	
19.....	(b)	6.41	
Average.....		6.57	±.116

<sup>a</sup> Samples of the bones of cattle numbered with the prefix WVa were furnished by H. O. Henderson of the West Virginia Agricultural Experiment Station.

<sup>b</sup> Mature.

TABLE 2.—*Calcium phosphate/calcium carbonate ratio in the skeletons of mature dairy cattle fed rations containing different levels of phosphorus*

ANIMALS ON A PHOSPHORUS-DEFICIENT RATION

Animal name or No.	$\frac{\text{Ca}_3(\text{PO}_4)_2}{\text{CaCO}_3}$	Standard deviation
Daisy.....	4.84	
E-36.....	5.36	
E-37.....	<sup>a</sup> 6.22	
E-73.....	5.66	
E-90.....	5.34	
Average.....	5.30	±0.294

ANIMALS ON A PHOSPHORUS-DEFICIENT RATION SUPPLEMENTED WITH SODIUM OR CALCIUM PHOSPHATE

E-34.....	6.30	
E-68.....	6.31	
E-59.....	6.20	
E-61.....	6.29	
E-62.....	6.77	
Average.....	6.37	±0.202

<sup>a</sup> Omitted from the average.

The animal called Daisy showed symptoms of extreme phosphorus deficiency. When she was slaughtered her ribs were found to be extremely soft and were readily pierced with a knife. E-90 was very emaciated, showed depraved appetite, and her joints creaked when she walked. The inorganic phosphorus in her plasma as determined by the Briggs (2) method was nearly 1.00 mg. per 100 c. c. of plasma. E-36 was in a similar condition. E-73 did not show such acute symptoms of phosphorus deficiency, but would chew bones. The inorganic phosphorus in her plasma averaged 2.00 mg. per 100 c. c. of plasma for the six months previous to slaughter.

Daisy, E-90, and E-36 showed the lowest ratios, E-73 somewhat higher, and E-37 a nearly normal ratio. This was in direct agreement with the degree of deficiency exhibited. The average ratio, 5.30, is almost the same as that reported for rachitic rats (6). The relative decrease in the amount of calcium phosphate indicates that there is a selective absorption during periods of deficiency. The animal in drawing on its mineral reserve in the skeleton for phosphorus either did not withdraw a proportionate amount of carbonate, or even replaced some of the calcium phosphate with calcium carbonate. The result may also indicate that the metabolism of the bone carbonates is independent of that of the phosphates.

It has been shown (3) that the feeding of the phosphorus-deficient ration supplemented with either sodium or calcium phosphate will result in normal reproduction, increased lactation, and gain in body weight. The bones of five animals so fed were analyzed. Before the feeding of the supplement the condition of these animals was comparable to that of the phosphorus-deficient group. E-62, which received the supplement longest, showed the highest ratio, 6.77. (Table 2.) All the ratios were within the range of the normal, and from this it is concluded that the skeletal structures of the animals had recovered their normal proportion of calcium phosphate and calcium carbonate. These are believed to be the first analyses reported from cattle after a period of phosphorus-deficiency followed in turn by adequate rations, and they indicate that recovery from such a condition can be complete.

Less is known of the effect of phosphorus deficiency on growing cattle than on mature ones. Henderson and Weakley (5) found an increase in the moisture content and a decrease in the breaking strength of bones from cattle raised on a low phosphorus ration. Bone samples from eight of their animals on low phosphorus rations are included in this study. (Table 3.) During the first year these animals received an ample quantity of phosphorus, but during the second year they were provided with only 60 to 75 per cent of the phosphorus usually retained.

TABLE 3.—*Calcium phosphate/calcium carbonate ratio in the skeletons of young dairy cattle fed rations containing different levels of phosphorus* <sup>a</sup>

ANIMALS ON A LOW PHOSPHORUS RATION			
Animal No.	Age	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> CaCO <sub>3</sub>	Standard deviation
		Months	
WVa-27.....	18	5.94	±0.201
WVa-30.....	18	6.12	
WVa-19.....	18	6.49	
WVa-22.....	18	6.11	
Average.....		6.16	
WVa-28.....	24	6.86	±.225
WVa-29.....	24	6.39	
WVa-20.....	24	6.92	
WVa-21.....	24	6.51	
Average.....	24	6.67	
ANIMALS ON A HIGH PHOSPHORUS RATION			
E-87.....	6	8.04	
E-90.....	6	7.65	
Average.....		7.85	

<sup>a</sup> The bone samples from animals on a low phosphorus ration were furnished by Doctor Henderson and Professor Weakley of the West Virginia Agricultural Experiment Station and represented samples used by them in another study (5). We acknowledge their kindness in supplying us with this material.

The calcium phosphate/calcium carbonate ratio was reduced in these animals. The normal ratios for cattle 18 and 24 months old were 7.05 and 7.16, respectively, as compared with 6.16 and 6.67 for these animals. Henderson observed some improvement in their condition during the last six months, and attributed it to a lessened phosphorus requirement which made a partial recovery possible. The ratios observed support this explanation of their improvement.

One-half of the animals of this group received a ration that was low in both calcium and phosphorus. There was no apparent ill effect from the low calcium, possibly because the deficiency of phosphorus reduced the requirement for calcium by preventing the usual amount of new calcification. The rations used were not so low in phosphorus as those employed at University Farm, nor were the ratios obtained so low. However, considering the difference in the normal ratios for the two ages, comparable reductions were secured in both cases. The higher mineral requirement of the young animal for skeletal growth makes it possible to secure a deficiency on rations carrying appreciable quantities of phosphorus.

It was planned to study the effect of low, medium, and high calcium and phosphorus intake on the growth and development of dairy cattle. Only two animals were available in the high phosphorus group, and none in the medium group when these data were obtained. (Table 3.) Both of the animals in the high phosphorus group were slaughtered at 6 months of age, and the high ratio of 7.85 was secured from the analyses of the bone samples. This raises the question whether an abnormally high reserve of phosphorus can be stored in the skeleton, and if so, would it not aid in meeting the phosphorus requirement during later lactation? It seems improbable that there should be any deleterious effects from high phosphorus intake in



view of the quantity of supplement (100 gm. of sodium or calcium phosphate daily) fed to cows on phosphorus-deficient rations (3).

The necessity of feeding milk to calves made it impossible to secure satisfactory low calcium rations for the first six months. Although the usual calcium intake was close to the amount generally retained, some departure from the normal was observed in three of the calves slaughtered at 6 or 12 months. (Table 4.) Three of those slaughtered at 18 and 24 months showed an increase in the calcium phosphate/calcium carbonate ratio, while the fourth was practically normal. However, there were too few animals and the calcium intake was not sufficiently low to warrant any definite conclusions. Indications are that either an increase or a decrease in the proportion of phosphate in the skeleton may occur as a result of calcium deficiency.

TABLE 4.—Calcium phosphate/calcium carbonate ratio in the skeletons of young dairy cattle fed rations containing different levels of calcium

ANIMALS ON A LOW CALCIUM RATION			
Animal No.	Age	$\frac{\text{Ca}_3(\text{PO}_4)_2}{\text{CaCO}_3}$	Standard deviation
	<i>Months</i>		
E-103.....	6	6.60	
E-104.....	6	6.91	
E-109.....	12	6.85	
E-110.....	12	7.23	
Average.....		6.90	±0.224
WVa-11.....	18	7.75	
WVa-14.....	18	7.76	
WVa-12.....	24	7.61	
WVa-13.....	24	7.27	
Average.....		7.60	±.198
ANIMALS ON A HIGH CALCIUM RATION			
E-95.....	12	7.58	
E-89.....	12	7.25	
Average.....		7.41	

There were but two animals on a high calcium ration. The ratio of calcium phosphate to calcium carbonate was normal when they were slaughtered at 12 months of age.

### CONCLUSION

The residual calcium/residual phosphorus ratio of cattle bone approximates the calcium/phosphorus ratio of tricalcium phosphate. The residual calcium/residual phosphorus ratio of the bone is not affected by calcium or phosphorus deficiency in the ration. This is the ratio between the calcium and phosphorus which do not occur in the bone as calcium carbonate or trimagnesium phosphate; it always closely approximates the value for tricalcium phosphate.

The calcium phosphate/calcium carbonate ratio of the bones of dairy cattle is affected by age and nutrition. This ratio decreases with age. Phosphorus deficiency causes a decreased ratio, but feed-

ing a phosphorus supplement permits the recovery of a normal ratio. Rations high in phosphorus may produce a high ratio.

There were indications that either an increase or a decrease in the proportion of phosphate in the skeleton may occur as a result of calcium deficiency, although the results did not warrant definite conclusions. The ratio of calcium phosphate to calcium carbonate was normal in two animals on a high calcium ration when slaughtered at 12 months of age.

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## A STATISTICAL STUDY OF THE RELATION BETWEEN VARIOUS EXPRESSIONS OF FERTILITY AND VIGOR IN THE GUINEA PIG<sup>1</sup>

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### PLAN OF THE STUDY

The primary object of the present study was to determine the relation between various measurable components of fertility and vigor with reference to the interval between the reaction of each to common environmental influences. The relationships were measured by simple correlations between the indices for eight characteristics which were calculated for 217 consecutive months (December, 1906, to December, 1924, inclusive) from the records on the inbred guinea pigs in the colony belonging to the Animal Husbandry Division, Bureau of Animal Industry of the department. Bulletins dealing with the effects of inbreeding by brother and sister matings in the colony have been published by Wright (16, 17)<sup>3</sup> and Wright and Eaton (18).

The size of the population studied, together with the distribution by size of litter and sex, is given in Table 1. The litter sizes in this stock varied from 1 to 8, averaging  $2.583 \pm 0.0041$  young per litter. The total number of individuals in each litter size was large except in litters of 7 and 8. It is of interest to note that 82.79 per cent of the entire population was born in litters of 2, 3, and 4. The sex of 391 individuals was not determined, and the birth weights of 243 individuals were not recorded.

### THE MONTHLY INDICES

Indices were calculated for each month from December 1906, to December, 1924, inclusive, for litters per 100 matings, average size of litter, sex ratio, average birth weight, average gain between birth and weaning, percentage of young born alive, percentage raised of those born, alive, and number raised per 100 matings.

<sup>1</sup> Received for publication Mar. 21, 1930; issued February, 1931.

<sup>2</sup> The results presented in this paper are taken from a part of a thesis offered to the University of Maryland in partial fulfillment of the requirements for the degree of doctor of philosophy. The data on which the paper is based are the property of the Animal Husbandry Division, U. S. Department of Agriculture, of which E. W. Sheets is chief, and the author wishes to express his sincere appreciation to the division for permission to make this analysis. He desires also to acknowledge his gratitude to Sewall Wright, collaborator of the division, for suggesting, directing, and commenting on the study, as well as to De Voe Meade, of the University of Maryland, who jointly supervised the work, and to H. C. McPhee and O. N. Eaton, of the Animal Husbandry Division, for assistance. Acknowledgment is also made for the work of Helena J. Haines, who calculated the correlation coefficients and checked many of the computations.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 163.

¶ The necessary steps and means of calculating each index, which also serve as definitions, were as follows:

1. Litters per 100 matings. The number of litters per 100 matings was the index indicating the frequency at which litters occurred and was calculated as one hundred times the number of litters born in a particular month divided by the number of pairs mated in that month. As nearly all matings were made between brothers and sisters at 33 days of age, when the males, at least, and usually both males and females, were sexually immature, a new mating was not counted until two months after it occurred. The basis for the correction was Wright's (15) findings that the minimum age at which males may sire litters is about 60 days, and the average age at which first litters are born is 5.9 months. Mature animals require some time to become acquainted in new matings. Matings were stopped in the month after that in which the female died and two months after the male died. The final value was corrected to a 30-day month.

2. Size of litter. The average size of litters was readily calculated from the total number of litters of all sizes and the number of individuals born in a particular month.

3. Sex ratio. Sex ratio was expressed as percentage of males rather than by the more customary means—the number of males per 100 females.

4. Birth weights and gains. Weights show a great deal of variation, which is in part related to the size of litter, mortality, and sex. The birth weights were calculated separately for each litter size, for each sex, and for those born dead, those dying between birth and weaning, and for those raised to weaning; but since gains up to weaning were likewise to be obtained, it was decided to use the average weights of those which were raised to weaning as the monthly index.

In calculating the gains, the average weaning weight (at 33 days) for each month, corrected for size of litter and sex, was first determined and the difference between the corrected birth weight and the corrected 33-day weight gave the gain for the month. The gains for a particular month referred to the calendar month in which the animal was born rather than to the month in which the gain was made; thus, the relation between birth weights and gains is not lost.

The basis for the corrections of the birth weights and gains to eliminate the effect of size of litter and sex is described later.

5. Percentage of young born alive and percentage raised. The percentage of young born alive and the percentage raised of those that were born alive are also affected by the size of litter, and a method for the elimination of this effect is described later. The months apply to the month of birth for the percentage raised, as in the case of gains.

6. Young raised per 100 matings. The young raised per 100 matings gives the relation between the actual number of young born in a particular month which were raised to weaning age and the number of pairs of parents mated in the same month. The month referred to is the month in which the individuals were born. This index was corrected to a 30-day month.

## EFFECT OF LITTER SIZE AND SEX ON WEIGHTS, GAINS, AND MORTALITY

Various investigators have found that the size of litter and the sex of young have a distinct influence on birth weights, gains to weaning, and mortality. The proportion of large litters also differs in different seasons. It was therefore necessary in studying the influence of season on these characteristics to determine first the influence of litter size and sex.

Because of the small numbers involved and their exceptional relations to the other litter sizes, litters of 7 and 8 were eliminated from the study of seasonal influences, but to make the data complete they are usually included in the tables giving averages for litter size. In the total population (Table 1) litters of 8 occurred at the rate of only 1 to nearly 4,000 litters, and litters of 7 occurred at the rate of 1 to each

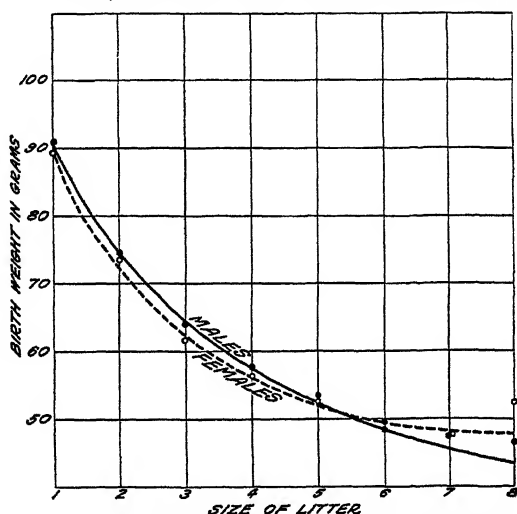


FIGURE 1.—Average birth weights of males and of females born dead in each litter size from litters of 1-6, fitted by second-order parabola, litters of 7 and 8 fitted by extrapolation. For males  $y = 0.008571 + 0.002592x - 0.000099x^2$ ; for females  $y = 0.008083 + 0.003255x - 0.000207x^2$ . Solid lines show birth weights of males and broken lines birth weights of females. Solid dots are observed values for males and circles observed values for females.

629 litters. The large litters also came in the most favorable seasons. Their omission from the calculations of the monthly indices could have no significant influence on the final results.

TABLE 1.—Number of litters and individuals included in the animals studied

Size of litter	Litters	Individuals	Born alive	Raised to weaning	Males *	Females *
	Number	Number	Number	Number	Number	Number
1	2,037	2,037	1,650	1,351	1,008	1,001
2	3,952	7,904	6,854	5,759	3,970	3,854
3	3,693	11,079	9,442	7,892	5,538	5,405
4	1,641	6,564	5,074	4,070	3,261	3,209
5	484	2,420	1,710	1,333	1,202	1,178
6	116	696	431	308	353	332
7	19	133	79	52	67	63
8	3	24	17	15	15	9
Total of 1-6	11,923	30,700	25,161	20,708	15,332	14,980
Total of all	11,945	30,857	25,287	20,775	15,414	15,052

\* Sex was not recorded for 391 individuals.

## BIRTH WEIGHTS

Wright (16, 17) pointed out that the birth weights of guinea pigs vary greatly, ranging from approximately 40 to 150 gm. for those that are raised. He also stated that size of litter was at least a very important contributory factor to the variability in weights, and found a correlation of  $-0.658 \pm 0.007$  between size of litter and mean birth weights of litter mates (15). Table 2, giving the average birth weights of the individuals in each of the three groups of males and females, according to size of litter, shows a definite relation of birth weight to size of litter. Notwithstanding the extreme variability of individuals, Figures 1 and 2 show that the average birth weights of those born dead, those dying between birth and weaning, and

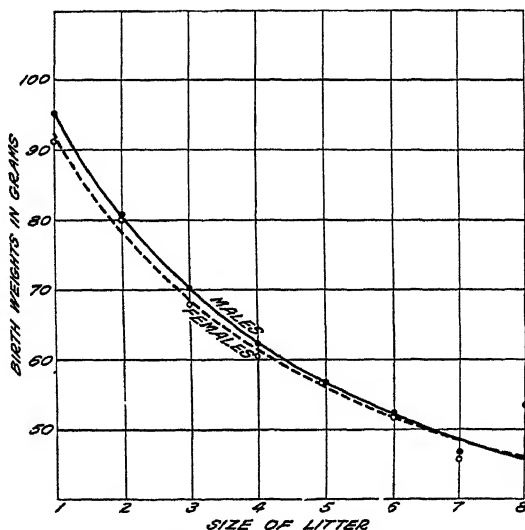


FIGURE 2.—Average birth weights of males and females dying between birth and weaning in each litter size fitted from litters of 1-6 by second-order parabola, litters of 7 and 8 fitted by extrapolation. For males  $y^2 = 0.008449 + 0.002088x^2 - 0.000051x^3$ ; for females  $y^2 = 0.008758 + 0.00214^2x - 0.000065x^3$ . Solid line shows birth weights of males and broken line birth weights of females. Solid dots are observed values for males and circles observed values for females.

those raised to weaning of each sex form relatively smooth and regular curves when plotted according to size of litter.

In all six groups the average birth weights of litters of 1 are decidedly the largest. A sharp downward trend in birth weights follows for litters of 2 and 3, but after this the weights decrease at a much slower rate. Litters of 7 and 8 show considerable irregularity, probably due to the small numbers.

The mortality of the newborn young in each litter size appears to be closely associated with the average birth weight. In litters of 1 the males raised weighed at birth nearly 13 gm. and the females 14 gm. more than those which died between birth and weaning. A difference of 8 to 10 gm. is maintained throughout the different litter sizes in both sexes with small fluctuations, except in litters of 8. There is a smaller but quite consistent difference in the birth weights of those which were born alive but died before weaning and those

which were born dead. The significance of the differences between the birth weights of the different groups was tested for males and females according to Student's method as described by Fisher, using litters of 1 to 6, inclusive (1, 2). Table 3 gives the data needed for interpretation.

TABLE 2.—Average weights of guinea pigs according to size of litter

MALES						
Size of litter	Birth weight of animals born dead	Birth weight of animals that died	Birth weight of animals raised to weaning	Weaning weight	Gain in weight to weaning	
	Grams	Grams	Grams	Grams	Grams	Per cent of birth weight
1.....	90.95	95.06	107.75	280.18	172.43	180.03
2.....	74.68	80.98	91.61	251.17	159.56	174.17
3.....	64.04	70.15	79.39	221.23	141.84	178.66
4.....	57.58	62.32	71.67	204.12	132.45	184.81
5.....	53.28	56.84	66.99	198.87	131.88	196.87
6.....	48.28	52.26	62.50	192.56	130.06	208.10
7.....	47.54	46.71	55.83	201.38	145.55	260.70
8.....	46.50	53.50	55.80	145.40	89.60	260.57
Average of 1-6.....	63.85	71.10	82.09	228.22	146.13	178.01
Average of all.....	63.65	70.93	82.02	228.12	146.10	178.13

FEMALES						
1.....	89.41	91.36	105.70	273.79	168.09	159.03
2.....	73.60	79.97	89.42	241.26	151.84	169.81
3.....	61.84	67.93	77.60	215.40	137.80	177.58
4.....	56.22	60.80	69.02	196.64	127.62	184.90
5.....	52.38	56.51	64.93	190.02	125.09	192.65
6.....	49.51	51.82	59.80	179.30	119.50	199.83
7.....	47.68	45.85	55.50	178.71	123.21	222.00
8.....	52.33	.....	64.50	141.00	76.50	118.60
Average of 1-6.....	62.78	69.42	79.92	220.48	140.56	175.88
Average of all.....	62.64	69.28	79.85	220.34	140.49	175.94

TABLE 3.—Comparison of birth weights of guinea pigs in groups according to fate

Groups compared	Mean differences	Standard deviation * of differences	t
	Grams	Grams	
Males born dead and those that died.....	4.80	1.154	10.19
Females born dead and those that died.....	4.24	1.847	5.63
Males died and males raised.....	10.38	1.252	20.31
Females died and females raised.....	9.68	2.382	9.96

$$* \sigma = \sqrt{\frac{\sum d^2}{n-1}}$$

With six litter sizes there are five degrees of freedom, and from Fisher's table of  $t$  (2), if  $t=4.032$ ,  $P$  is 0.01. Since all values of  $t$  observed exceed 4.032, there is less than 1 chance to 99 that the differences are not significant. It must, therefore, be concluded that the average birth weights of the different groups differ significantly in both sexes.



Slight differences in the shape of the curves for the different groups are apparent in both sexes, but the close approach to parallelism in the two sexes, with the males slightly heavier in all groups and in nearly all litter sizes, is beyond expectation. This is best noted in Figures 1, 2, and 3. Because of the uniformity of the differences within different litter sizes, males would appear to be heavier than females at birth, but the average difference between males and females in litters of 1-6 for those born dead was 0.98 gm., and for those dying between birth and weaning, 1.54 gm. The respective standard deviations of the differences were 1.170 and 1.272 gm., respectively, from which the values of  $t$  were calculated as 2.05 and 2.96. The corresponding  $P$  values were 0.1 and 0.04. Thus the sex difference in the birth weights of those born dead is not significant, and for those

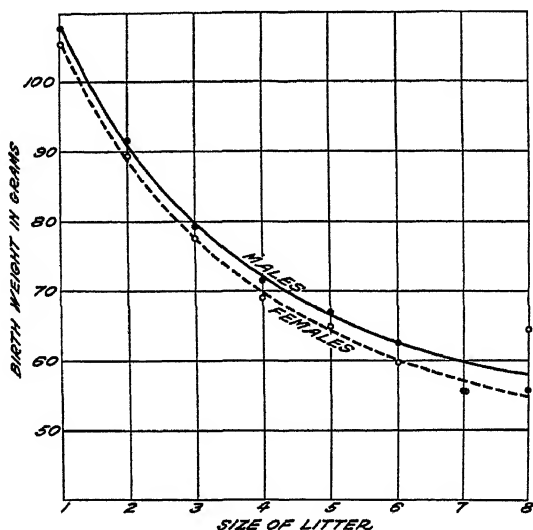


FIGURE 3.—Average birth weights of males and females raised to weaning in each litter size fitted from litters 1-6 by second-order parabolas, litters of 7 to 8 fitted by extrapolation. For males  $y = 0.007303 + 0.002050x - 0.000101x^2$ ; for females  $y = 0.007441 + 0.002089x - 0.000092x^2$ . Solid line shows birth weights of males and broken line birth weights of females. Solid dots are observed values for males and circles observed values for females

that died it can not be considered certain. The birth weights of males raised are also greater than the birth weights of females raised; the mean difference in litters of 1-6 was 2.24 gm. with  $\sigma$  of 0.361 gm. making  $t$  15.20 and  $P$  much less than 0.01. With five degrees of freedom, when  $t$  is 4.032,  $P = 0.01$ .

It may be concluded that birth weights are significantly related to size of litter and to mortality of young before and after birth, and that the birth weights of those individuals which are raised, as well as those which die between birth and weaning, are related to sex.

#### WEANING WEIGHTS AND GAINS

The effects of sex and size of litters on weaning weights and gains from birth to weaning are brought out in Table 2. Figure 4 shows a pronounced effect of sex on weaning weight similar to that on birth weight. Males are heavier in all litter sizes. The relation of size of

litter to weaning weight is also similar to the relation of size of litter to birth weight, especially in the small litter sizes.

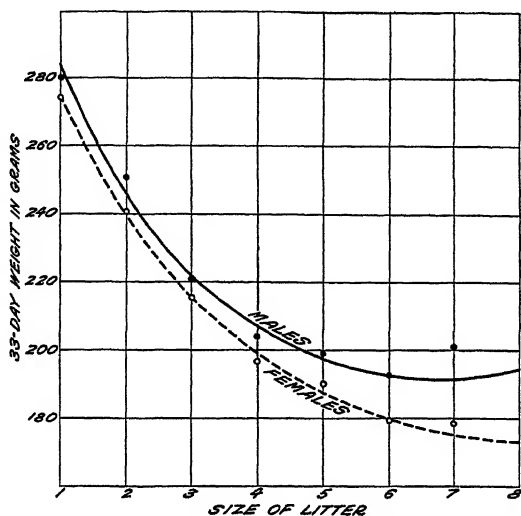


FIGURE 4.—Average weights of males and females at 33 days of age (weaning) born in each litter size fitted, from litters of 1-6, by second-order parabola, litters of 7 and 8 fitted by extrapolation. For males,  $y = 0.0028884 + 0.0006922x - 0.0000514x^2$ ; for females,  $y = 0.0030235 + 0.0006550x - 0.0000388x^2$ . Solid line shows weaning weights of males, and broken line weaning weights of females. Solid dots are observed values for males and circles observed values for females.

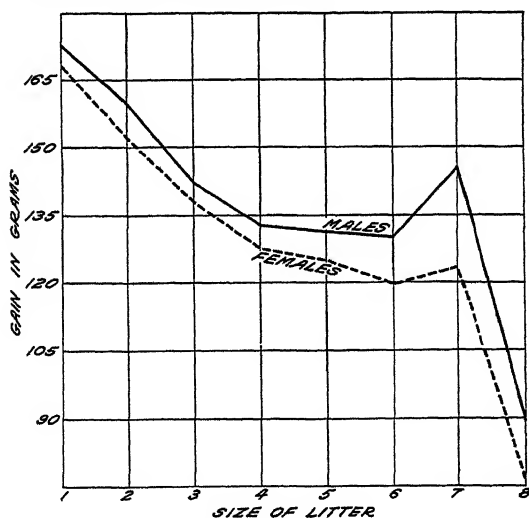


FIGURE 5.—Average gains between birth and weaning (33 days) for males and females born in different-sized litters. Solid line shows gains of males and broken line gains of females.

The mean difference between the weaning weights of males and females in litters of 1-6 was 8.62 gm. and the standard deviation of the difference was 2.731 gm.;  $t$  was, therefore, 7.73, and with five degrees of freedom,  $P$  is considerably less than 0.01.

The weaning weight is a combination of the birth weight and the gain made between birth and weaning. Figure 5 shows the influence of sex and litter size on the gains between birth and weaning.

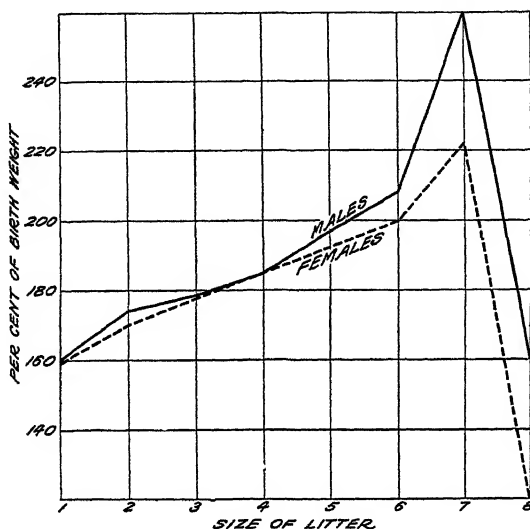


FIGURE 6.—Gain between birth and weaning (33 days) of males and females born in the different-sized litters, expressed as percentage of birth weight. Solid line shows percentage gain for males and broken line percentage gain for females

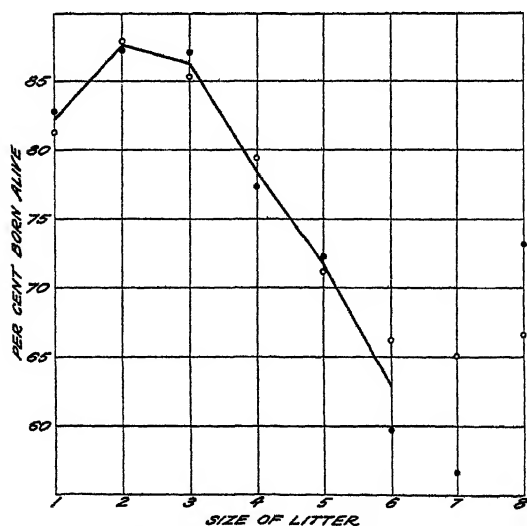


FIGURE 7.—Percentage of males and females born alive in the different litter sizes. Solid line shows the mean percentage for the two sexes in litters of 1-6, solid dots the percentage of males born alive, and circles the percentage of females born alive in each litter size

though it might be assumed that in an animal born as mature as the guinea pig and which is so little dependent on its dam's milk supply (5), the size of litter would have practically no effect on postnatal

growth, it is very apparent that, whether due to the care and food supplied by the dam or to their larger birth weights, the young born in the small litters have a distinct advantage in the amount of gain. The percentage increases in weight during the weaning period (Table 2 and fig. 6) are conversely related to birth weights. The increase for males in litters of 6 was calculated at 208.10 per cent, but in litters of 1 it was 160.03 per cent, with comparable values for females. Thus an increased relative gain accompanies an increase in litter size and tends somewhat to offset the percentage differences between the weights at birth in the different sized litters. Considering the

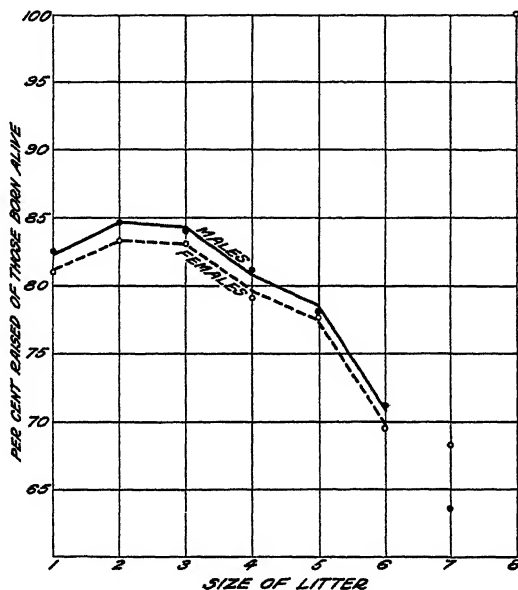


FIGURE 8.—Percentage of males and females born alive which were raised to weaning in each litter size. Solid line shows the percentage of males and the broken line the percentage of females in each litter size from 1-6 as calculated from the mean of the two sexes by the method of weighing explained in the text. Solid dots give observed percentages for males and circles observed percentages for females in each litter size

small numbers involved, litters of 8 can hardly be regarded as a reliable exception.

The mean difference between the gains of males and females in litters of 1-6 was 6.38 gm. with a standard deviation of 2.509 gm., giving a value for  $t$  of 6.03; with five degrees of freedom,  $P$  is therefore less than 0.01.

The weaning weights and gains between birth and weaning of males were thus significantly greater than those of females.

#### MORTALITY OF YOUNG

A study of Table 4, which gives the number of animals born dead, the number that died, and the number raised of each sex in litters of different sizes, shows that size of litter bears an important relationship to mortality. The percentage of young born alive is shown in Figure 7, and the percentage raised of those that were born alive is shown in Figure 8.

TABLE 4.—Number and mortality of males and females born in each litter size

Size of litter	Males					Females				
	Born dead	Died	Raised to weaning	Born alive	Raised to weaning of young born alive	Born dead	Died	Raised to weaning	Born alive	Raised to weaning of young born alive
	Number	Number	Number	Per cent	Per cent	Number	Number	Number	Per cent	Per cent
1.....	173	145	690	82.84	82.63	186	154	661	81.42	81.10
2.....	503	533	2,934	87.33	84.63	467	562	2,325	87.88	83.41
3.....	715	772	4,051	87.09	83.99	786	778	3,841	85.46	83.16
4.....	737	473	2,051	77.40	81.26	659	531	2,019	79.46	79.18
5.....	332	190	680	72.38	78.16	339	187	653	71.25	77.74
6.....	142	61	150	59.77	71.09	112	67	153	66.27	69.55
7.....	29	14	24	56.72	63.16	22	13	28	65.08	68.29
8.....	4	2	9	73.33	81.82	3	-----	6	66.67	100.00
Total of 1-6.....	2,602	2,174	10,556	*83.03	*82.92	2,549	2,279	10,152	*82.98	*81.67
Total of all.....	2,635	2,190	10,589	*82.91	*82.86	2,574	2,292	10,186	*82.90	*81.63

\* Weighted average.

The one very striking feature of these data is that the chances of survival for individuals born in litters of 1 are less than for those born in litters of 2 and 3. It has been observed by Wright (16, 17) that frequently very large animals are born dead, evidently due to difficulties at parturition. Such difficulties might have had some influence on the smaller percentage born alive in litters of 1, but would in no way explain the lower percentage raised of those born alive in this litter size. The explanation for the higher mortality in litters of 1 is probably the lowered vigor of dams producing litters as small as 1.

The chances of the animals being born alive in litters of 4, 5, and 6 decrease very rapidly with each increase in litter size. The fact that the curve for the percentage raised of those born alive does not drop off nearly so rapidly with larger litters as the curve for percentage born alive indicates the lesser dependence on the dam and is, no doubt, also a result of the reduced size of the larger litters through heavier mortality at birth.

It is very apparent that the difference in mortality between the sexes is less than the difference in weights. The percentages of the two sexes born alive alternate at each litter size, indicating that there is no significant difference between males and females in this respect; the weighted averages were 83.03 per cent for males and 82.98 per cent for females in litters of 1-6.

There is a slight but constantly higher percentage of males than of females raised in litters of 1-6 (fig. 8), the mean weighted averages being 82.92 per cent for males and 81.67 per cent for females. The percentages raised in the different litter sizes indicated a sex difference in this characteristic.

To test the likelihood of the distribution of males and females within normal expectation,  $\chi^2$  was determined for fourfold tables for each litter size from 1-6, according to the method outlined by Fisher (2).

The values of  $\chi^2$  and  $P$  are given in Table 5. The  $\chi^2$  values in litters of 3 and 4 indicate the distributions of the sexes among the young born dead and those born alive which would occur less than 5

times in 100 trials if there were equal mortality of the two sexes. There were more males than females born alive in litters of 3 (87.09 per cent males and 85.46 per cent females), and more females than males born alive in litters of 4 (79.46 per cent females and 77.40 per cent males). The alternation of the two sexes in these and the other litter sizes, indicate that there was no difference in the mortality of the two sexes at birth, even though the deviation from equality in the litter sizes would be expected to occur but about twice in 100 times. None of the  $\chi^2$  values for the animals that died and those that were raised are without the normal expectation.

TABLE 5.—*Chi-square test of the distribution of the sexes according to fate*

Size of litter	Born dead and born alive		Died and raised to weaning	
	$\chi^2$	P	$\chi^2$	P
1	0.689	0.30-0.50	0.651	0.30-0.50
2	0.550	.30-.50	1.898	.10-.20
3	6.150	.01-.02	1.204	.20-.30
4	4.074	.02-.05	3.469	.05-.10
5	3.777	.05-.10	.045	.80-.90
6	3.090	.05-.10	.123	.70-.80
Total N=6..	14.930	.02-.05	7.390	.20-.30

Since males uniformly excel females in the percentages raised in litters of 1-6, Student's method (1, 2) affords a test for the significance of the difference. Comparison was, therefore, made of the percentage of males and of females raised in litters of 1-6. The mean difference in the percentage raised was 1.27 per cent in favor of the males, with a standard deviation of 0.586 per cent. Thus  $t$  was 5.31, for which  $P$  with five degrees of freedom is less than 0.01. This difference is, therefore, clearly significant.

In litters of 1-6 the percentages of males in the different groups were as follows: In the group born dead,  $50.145 \pm 0.470$  per cent,<sup>4</sup> died  $48.821 \pm 0.505$  per cent, and raised  $50.976 \pm 0.2641$  per cent. The difference between the percentage of males among those born dead and died was  $1.6935 \pm 0.6901$  per cent, and between those died and raised  $2.1545 \pm 0.5568$  per cent.

The data presented show that the size of litter in which young are born bears an important relation to mortality at birth and between birth and weaning, litters of 2 and 3 being most favorable for survival. Sex had no influence on mortality at birth, but between birth and weaning the mortality of females was slightly greater than that of males.

#### SEX RATIO

Among all young born there were  $50.5941 \pm 0.1929$  per cent males, a probably significant departure from 50 per cent. The percentages of males in litters of 1, 2, 3, 4, 5, 6, 7, and 8 were 50.17, 50.74, 50.61, 50.42, 50.48, 51.53, 51.54, and 62.50 per cent, respectively. The  $\chi^2$  test for the departure of the sex ratio in the different litter sizes (1-8) from the mean ratio does not give support to the significance of the departures.  $\chi^2$  was 2.0056. With  $n=7$ ,  $P$  is 0.96. Departures as

<sup>4</sup> Probable error for percentage of males equals  $0.6745 \sqrt{\frac{\text{Per cent males} \times \text{per cent females}}{n}}$

great as these from the mean are thus to be expected 96 times in 100 trials.

The significance of the deviations from equality of the sexes was tested by Fisher's *t* method, using only litters of 1-6 in which the numbers were relatively large. The mean difference was 1.317 per cent, with a standard error of  $\pm 0.3823$ , making *t*, 3.44—a value to be expected but about twice in 100 trials if there were no difference. If litters of 7 and 8 were included, the probability would be less.

#### DISCUSSION

Many authors have called attention to the effects of litter size on birth weights, including Gates (3) and Parkes (13), in mice, Hanson and Heys (4), in the rat; Kopéc (6, 7), in the rabbit; and Prawocheński and Kaczkowski (14), in sheep and other animals. Minot (11) made a study of the growth of guinea pigs from birth to maturity. He found that the size of litter had an important influence on birth weights, especially in the smaller litters. The differences in the birth weights he attributed mainly to differences in the length of gestation periods, but Wright (15) found that size of litter was much more effective in reducing birth weights by reducing the rate of growth than by causing early parturition. Differences in location or relation to other fetuses in the uterus may also influence the differences in the size of individuals in the same litter. Marshall (10) has discussed the possible effects of limited nutrition of the dam, mainly with negative conclusions. When the animals are more mature at parturition and the numbers of young are relatively small, the effect of additional numbers seems particularly important.

In the present study the birth weights, weaning weights, and gains of the two sexes conformed with the general findings of others in almost all classes of animals, males being generally heavier.

It is more difficult to find an explanation of the effect of size of litter on mortality and especially on prenatal mortality. The operation of lethal factors would be equally important in all litter sizes and, therefore, can not be considered. Nutrition might be a limiting factor, provided the young became very much weakened from lack of food, which is quite likely to have been a factor, as is indicated by the similarity between the birth weights of those dying between birth and weaning and those dying at birth as contrasted with the considerably greater average weight of those raised, but the condition and age of the dam and maturity of the young seem to be more likely possibilities. Here again the dam's influence particularly seems to be somewhat complicated. How can a female in sufficiently good condition to produce a litter of 5, 6, or 7 be considered in too poor condition to raise as large a proportion of her young as a female producing a litter of 2 or 3? The explanation is possibly related to the maturity of the young at birth. Because of the greater mortality at birth in large litters the actual number which the dam has to care for is not very much greater than the number cared for in smaller litters. Maturity alone, however, can not be the whole story, for it is well known and has been pointed out by Minot (11) and Wright (16, 17) that litters of 1 are most mature at birth, and yet both the percentages that are born dead and those that die between birth and weaning are larger in litters of 1 than in litters of 2 and 3. It may be that the explanation of the effect of litter size on mortality is a combination

of the maturity and competition of the young and the condition and age of the dam. The influence of the dam may apply particularly to litters of 1, whereas the maturity of the young is perhaps more important in large litters.

The sex ratios combined with mortality do not indicate large inequalities in the mortality of the two sexes at the different stages of development. The small differences during the weaning period have been pointed out. Much has been published on the sex ratios of animals, in many cases showing sex differences in mortality, the immature male usually being less vigorous. In the present study, however, females if anything were less viable during the period between birth and weaning.

#### ELIMINATION OF THE EFFECT OF LITTER SIZE ON WEIGHTS

The primary purpose of determining the average weights of males and females born in litters of different sizes was to get at some means of eliminating such effects in the calculation of monthly indices. The averages when plotted, especially in litters of from 1 to 6, formed relatively smooth curves. A very close fit to practically all the observations for birth weights and the 33-day weights was obtained for males and females in the different groups by fitting to the formula  $y^{-1} = a + bx + cx^2$ , in which  $y^{-1}$  represents the reciprocal of the weight in any litter size, and  $x$  in litters from 1 to 6; and  $a$ ,  $b$ , and  $c$  are constants. These values for males and females in the different groups are given in Table 6. The fitted curves for each group are shown in Figures 1, 2, 3, and 4. The solid line in each case represents the calculated curve for the weights of the males and the broken line the same for the females. Each observed average value is given as a solid dot for males and a circle for females.

TABLE 6.—Calculated values of constants in parabola<sup>a</sup>  $y^{-1} = a + bx + cx^2$

Group	<i>a</i>	<i>b</i>	<i>c</i>
Birth weight of males born dead.....	0.008571	0.002592	-0.000099
Birth weight of females born dead.....	.008083	.003255	-.000207
Birth weight of males that died.....	.008449	.002088	-.000051
Birth weight of females that died.....	.008758	.002142	-.000085
Birth weight of females raised.....	.007303	.002050	-.000101
Birth weight of females raised.....	.007441	.002089	-.000092
Weaning weight of males.....	.002884	.0006922	-.0000514
Weaning weight of females.....	.0030235	.0006550	-.0000388

<sup>a</sup>  $y$  is weight,  $x$  is size of litter and  $a$ ,  $b$ , and  $c$  are constants.

Correction factors for the weights of individuals born in any litter size were then calculated for each group from the formula  $C_x = \frac{M}{y}$ , where  $C_x$  represents the correction factor for litter size,  $x$ ;  $M$  is the mean weight of the group including all-sized litters; and  $y$  is the calculated average weight of the individuals born in a certain litter size,  $x$ . The reciprocals of the calculated weights for each litter size ( $y^{-1}$ ) were multiplied by the mean of the group to obtain the weight-correction factors given in Table 7.

The correction factors were calculated from the averages for litters of 1-6. In all groups the curves have a tendency to turn up



at or near litters of 7 or 8, thus showing heavier weights for the individuals in larger litters. This is not at all in conformity with the general tendencies observed where the numbers are sufficient, though in certain cases the curves appear to fit the observed data more closely because of turning up. Litters of 7 and 8 were not included in the calculation of monthly indices for weight and mortality.

TABLE 7.—Correction factors for size of litter

Item	Correction factor when size of litter is—						Average used as standard
	1	2	3	4	5	6	
							<i>Grams</i>
Birth weight of males born dead.....	0.7065	0.8530	0.9869	1.1082	1.2168	1.3128	63.8537
Birth weight of females born dead.....	.6988	.8642	1.0036	1.1169	1.2043	1.2657	62.7810
Birth weight of males that died.....	.7455	.8831	1.0134	1.1365	1.2523	1.3609	71.0971
Birth weight of females that died.....	.7521	.8873	1.0134	1.1305	1.2386	1.3377	69.4177
Birth weight of males raised.....	.7595	.9029	1.0298	1.1400	1.2337	1.3108	82.0935
Birth weight of females raised.....	.7543	.8992	1.0294	1.1449	1.2456	1.3317	79.9204
Weaning weight of males.....	.8054	.9282	1.0276	1.1035	1.1559	1.1849	228.2170
Weaning weight of females.....	.8025	.9212	1.0228	1.1074	1.1748	1.2251	220.4750
							<i>Per cent</i>
Per cent born alive.....	1.0107	.9476	.9621	1.0585	1.1558	1.3193	83.01
Per cent raised of those born alive.....	1.0051	.9795	.9847	1.0261	1.0558	1.1707	82.30

#### ELIMINATION OF THE EFFECT OF LITTER SIZE ON MORTALITY

The correction factors for mortality were not so easily smoothed as those for the weights. After a number of different methods and formulas had been tried, the most satisfactory method seemed to be to strike the mid-point between the values determined for males and females. The reciprocals for the combined percentages of males and females born alive, multiplied by the average for all-sized litters of 1-6, were used as the correction factors for percentage born alive. As no difference between the sexes was established, a single correction value was used for each litter size.

The basis for the correction factors for the effect of litter size on percentage raised was selected as the mid-point between the percentage of males raised and the percentage of females raised, since there were nearly equal proportions of the two sexes among those raised. These points were then modified by the average of one-half the weighted difference between the males and females at each point, and thus parallel curves were constructed. This procedure was carried out only for litters of 1-6 and appeared quite satisfactory.

The mortality correction factors based on the reciprocals of the smoothed values multiplied by the average for litters of 1-6 are also given in Table 7 for the percentage of males and females born alive and raised.

#### SEASONAL FLUCTUATIONS IN MONTHLY INDICES

All data were tabulated by months, litter size, mortality, and sex. After necessary corrections had been made for size of litter and sex, the means for weight and mortality were used as the indices for each month.

The monthly indices so calculated are presented in Figure 9. The values for the indices are given in the left-hand margin of the figure,

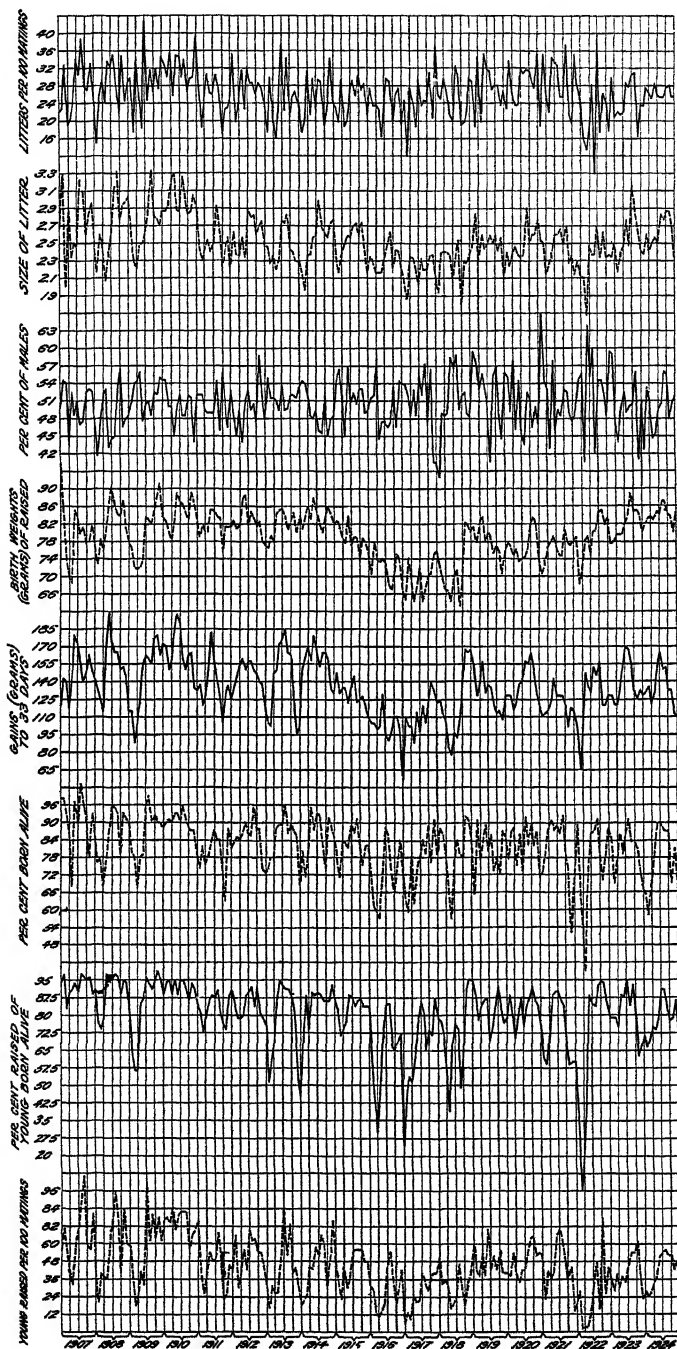


FIGURE 9.—Monthly indices calculated as explained in the text for litters per 100 matings, size of litter, percentage of males, birth weights of those raised to weaning, gains to weaning, percentage born alive, percentage raised of those born alive, and young raised per 100 matings. The vertical lines represent 3-month intervals

while the vertical lines represent the months from December, 1906, to December, 1924, inclusive, in 3-month intervals. In considering any of the series of data the fact that the vigor of the stock was undergoing a decline throughout the period should not be overlooked (Wright (16, 17)). It is also of general interest in this connection that conditions were very favorable in 1910 and rather unfavorable for a period from about 1915 to 1918.

#### LITTERS PER 100 MATINGS

The division of the time into calendar months proved rather inappropriate for the calculation of this index, as it gave a graph of a rather distinct zigzag or saw-tooth character, resulting from the fact that females producing litters in one month can not produce litters again in the next month because of the 68-day gestation period. Evidently when conditions become favorable the reaction is pronounced and litters are produced by a large portion of the females, which automatically prevents litters from being born to these individuals in the next month. The high and low points of the different years show some tendency toward seasonal effects, but there is much irregularity. There appears to be some tendency for the period from May to August to be a little more favorable with relatively high points in the fall in many years. It is a striking fact that the really high points in the late fall or early winter appear to be related to years in which no high point was observed in a spring or summer month.

It is evident that this index was not entirely satisfactory as a measure of the frequency at which litters were produced. Frequency of litters, being obviously a much-delayed evidence of conditions during a period of over two months prior to the time of parturition, tends further to complicate its expression.

#### SIZE OF LITTER

As in the case of frequency of litter, size of litter is mainly determined at conception, 68 days before the litter is born. There are, however, influences acting throughout pregnancy which may tend to reduce the size of the litter. In the case of this index there is apparently some cyclic behavior related to the season. From January to April the litter size is generally small, while from June to November it is usually larger. A few years like 1917, 1918, and 1919, however, show considerable irregularity and do not bear this out well.

#### SEX RATIO

The percentage of males shows much seasonal variation, but even with the irregular character of the graph there appears to be a slight tendency for the ends of the years to be high with a drop during the spring and summer months. The exceptions to this are so frequent, as in 1918, that this index can not be considered as following the season closely. Many years indicate that these variations are related to the behavior of the other indices. For instance, in 1909 practically all the other indices show a relatively high point in January with a drop in March and a rise to July, while the percentage of males goes in the opposite way. Again in 1920, 1921, and 1922 a similar relation is striking, but other cases do not bear this out.

In a test of the goodness of fit of the sex ratio in the individual months to the monthly average sex ratio observed, 50.58 per cent

males,  $\chi^2$  was found to equal 173.65.  $\chi^2$  tables do not go as high as  $n=216$  (217 months were used), but Fisher (2) suggests the use of  $\sqrt{2\chi} - \sqrt{2n-1}$ , in which differences significantly greater than 2 indicate that the data do not conform to expectation. Substituting for these values,  $18.64 - 20.76 = -2.12$ , which is in close agreement with expectation. Such is to be expected since almost all of the  $\chi^2$  values for individual months were low, only three causing any suspicion of significant departures from expectation, October, 1912, July, 1919, and January, 1921, in which the  $\chi^2$  values were respectively 6, 4, and 8. It is, of course, possible that variations in the sex ratio might be associated with the season and still such might not show wider departures from normal expectation than would be expected by chance.

#### BIRTH WEIGHTS AND GAINS

The birth weights are expressed at birth and gains are expressed 33 days after birth, but both are shown in Figure 9 for the month in which the young were born. The variations shown in the figure were similar in most years. This is probably due to the fact that the gains made in a certain month are evidently affected by the same influences that act on the fetus in utero and affect the birth weights of the individuals born in that month, although there is some indication that birth weights are a little slower in reacting than gains.

Both birth weights and gains show a rather regular cycle over most of the years, the low points being in the winter and the high points in the late spring or early summer (May and June). The years of unfavorable conditions, 1915, 1916, 1917, and 1918, are particularly prominent in these data. With some zigzagging, birth weights and gains show a rapid decline beginning approximately with December, 1914. In the favorable year, 1910, the customary drop in the late winter and early spring was much abbreviated.

#### MORTALITY

As in the case of the weights and gains, the percentage of animals born alive and the percentage raised of those that were born alive are recorded for the month in which the individuals were born, and are closely related, generally showing low points in the winter or early spring and high points in the summer or fall. In April the curve for percentage born alive was frequently low, while in July it was high. As in the gains, the percentage raised is affected by the same conditions that affect the percentage born alive; i. e., while the young are in utero the mortality is increased in the same way that the mortality of individuals between birth and weaning is affected. The fluctuations in the percentage raised are somewhat greater than the fluctuations in the percentage born alive, probably because for young in utero the dam acts somewhat as a buffer to immediate changes in the environment.

#### NET FERTILITY

The net fertility is a combination of four of the other characters and therefore should show somewhat the same general course. It is essentially the product of the litters per 100 matings, size of litter, percentage born alive, and percentage raised of those born alive, except that the two latter indices were corrected to eliminate the effects

of size of litter and sex. The general course of the net fertility curve is low late in the winter and early spring, reaching a peak in midsummer or early fall.

#### AVERAGES FOR COMBINED MONTHS

In the study of the seasonal relations of the indices, the data for all months of January, all months of February, etc., were combined and treated as one year. This was virtually the weighted averages for each of the months. In this way the indices for each of the combined months are based on large numbers of individuals, the smallest number in any month being 630 litters and 1,973 individuals in February. The records for the combined months are given in Tables 8 and 9, together with comparisons for the averages calculated, corrected and uncorrected weights, and mortality percentages being used. This was done for the purpose of estimating the effect of the correction factors used on the several groups of data. As is quite evident, eliminating the effect of size of litter and sex on weights and mortality has had little effect on the relative ranking of different months for a particular index, but it has tended to bring out more strikingly the variations.

TABLE 8.—Averages for fertility characters in combined months, 1906 to 1924 (litters of 1-6)

Month	Matings	Litters	Guinea pigs	Litters per 100 matings	Size of litter	Sex ratio	Born alive		Raised to weaning of those born alive	
							Cor- rected	Uncor- rected	Cor- rected	Uncor- rected
	Number	Number	Number	Number	Number	Per cent Males	Per cent	Per cent	Per cent	Per cent
January.....	3,738	979	2,400	26.19	2.45	52.45	78.28	79.45	73.60	74.03
February.....	3,692	830	1,973	22.48	2.38	52.19	76.87	78.01	69.22	69.62
March.....	3,695	1,055	2,563	28.55	2.43	51.79	77.60	78.00	71.02	71.40
April.....	3,581	844	2,085	23.50	2.47	47.76	80.38	81.11	82.00	82.35
May.....	3,491	904	2,224	25.90	2.46	50.52	85.21	85.57	87.67	87.85
June.....	3,541	1,061	2,734	29.96	2.58	50.48	88.12	87.99	90.06	90.03
July.....	3,540	1,053	2,924	29.75	2.78	49.41	90.08	88.53	88.88	88.20
August.....	3,632	1,076	3,032	29.63	2.82	49.60	87.00	85.40	85.87	85.27
September.....	3,651	919	2,405	25.17	2.62	52.47	88.65	83.52	85.53	85.48
October.....	3,773	1,094	2,916	29.00	2.67	51.32	81.26	80.83	83.07	82.88
November.....	3,917	1,028	2,715	26.24	2.64	49.03	84.26	84.36	83.83	83.89
December.....	3,899	1,080	2,729	27.70	2.53	50.39	79.66	80.27	78.80	79.09
Average.....	44,160	11,923	30,700	27.00	2.57	50.58	83.01	83.01	82.30	82.80

\* Total.

Size of litter tends to affect the influence of other factors on weights and mortality; for instance, if conditions are unfavorable litters will be small in size, but as a result of this birth weights will be correspondingly larger, etc. The elimination of the effects of sex has been relatively unimportant because the sexes were nearly equally distributed in the combined months, but this no doubt had an important effect in individual months where the numbers were smaller and the sex ratios showed more variation. The data for the combined months, as presented in Figure 10, afford a better opportunity for studying the average variations of the different indices with the seasons than does Figure 9, which shows the variations over the entire period.

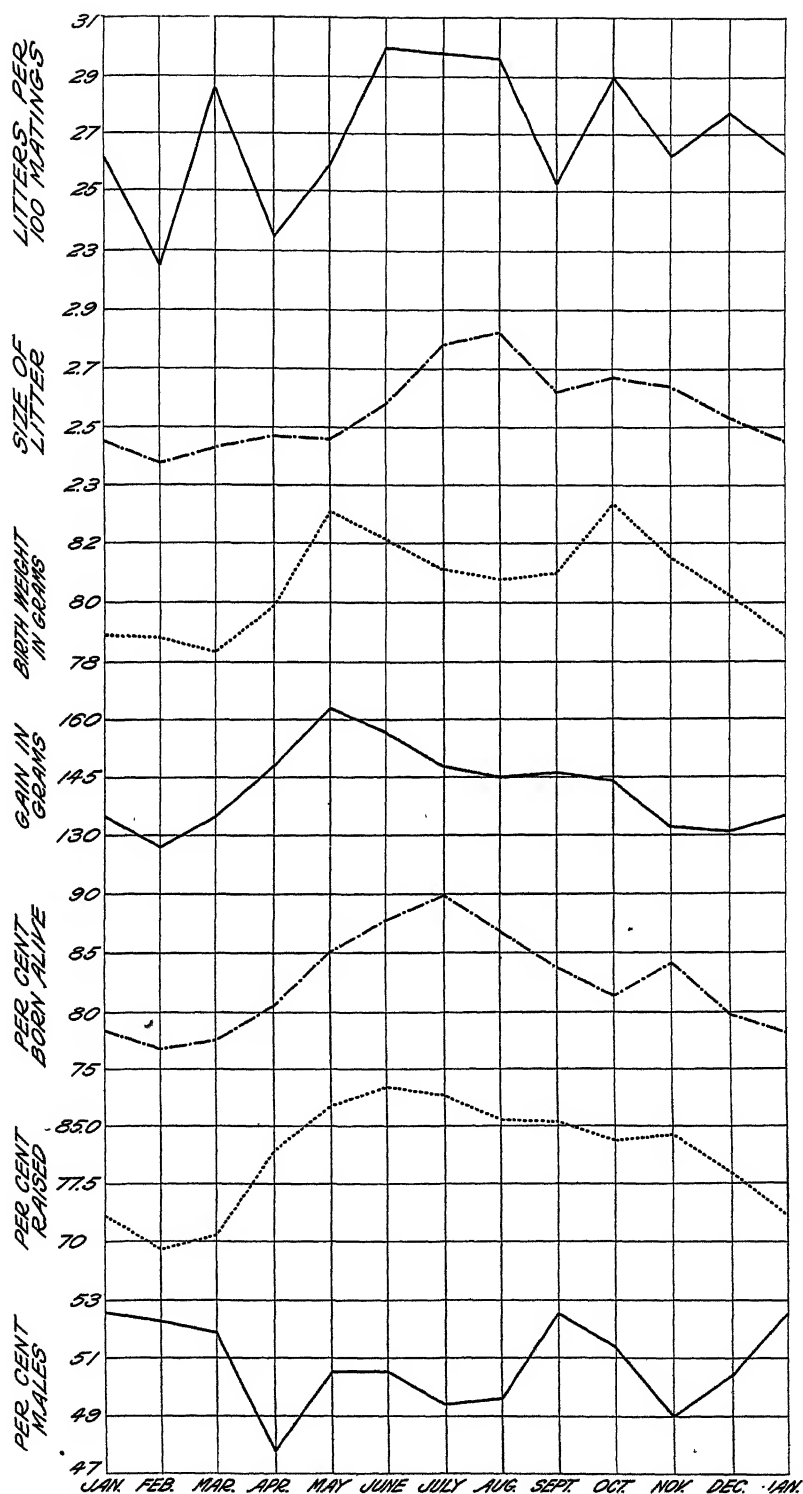


FIGURE 10.—Monthly indices for combined months (litters of 1-8). The month of December gives

TABLE 9.—Averages for birth and weaning weights and gains in combined months, 1906-1924 (litters of 1-6)

Month	Birth weights of animals—						Weaning weights		Gains in weight to weaning	
	Born dead		That died before weaning		Raised to weaning					
	Cor- rected	Uncor- rected	Cor- rected	Uncor- rected	Cor- rected	Uncor- rected	Cor- rected	Uncor- rected	Cor- rected	Uncor- rected
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
January.....	59.57	59.82	70.02	72.19	78.91	80.67	214.17	217.22	135.26	136.55
February.....	61.13	64.17	69.58	71.81	78.76	80.99	206.67	210.74	127.91	129.75
March.....	59.62	61.20	68.28	70.14	78.30	80.23	213.54	217.03	135.24	136.80
April.....	58.53	60.52	67.36	67.85	79.94	80.76	227.64	228.57	147.70	147.81
May.....	67.07	69.59	72.38	72.55	83.08	83.85	245.80	247.07	162.72	163.22
June.....	66.07	66.14	70.52	69.35	82.24	82.13	238.75	238.29	156.51	156.16
July.....	67.03	62.86	66.99	62.51	81.22	79.61	229.49	226.09	148.27	146.48
August.....	65.56	64.20	69.14	65.81	80.77	78.46	226.03	221.19	145.26	142.73
September.....	67.38	65.98	72.73	72.16	81.00	80.74	226.82	226.19	145.82	145.45
October.....	63.55	62.64	72.97	72.12	83.39	82.46	227.51	225.73	144.12	143.27
November.....	65.37	64.48	72.08	71.26	81.64	81.43	213.58	213.06	131.94	131.63
December.....	61.86	61.82	72.08	72.68	80.28	81.42	211.01	213.18	130.73	131.76
Average.....	63.25	63.32	70.26	70.24	81.01	81.03	224.62	224.42	143.61	143.39

It is indeed interesting that litters per 100 matings in the combined months show somewhat the same tendency to alternate in successive months as was observed to a greater extent in the individual months. For the data of the combined months the only case in which the alternating relationship is not shown in successive months is during the summer in June, July, and August, the most favorable season. The beginning of this effect is in May. All other months alternate.

Size of litter for the 6-month period, December to May, inclusive, is below the average, while the six months, June to November, are above the average. The high points for size of litter are in July and August, which appear to be considerably more favorable than other months.

The birth-weight curve is distinctly bimodal, showing high points in the spring and in the fall. Taken together, May and June form the highest mode. The second mode which is the highest for any one month is in October. The tendency for birth weights to rise in the fall was also evident in the curves for the individual months.

The gains showed much the same variation over the year as birth weights, except that the peak in the spring was much more pronounced, and there was but slight evidence of the fall mode. The maximum gains were made by those born during the month of May.

The mortality curves were similar in shape. Both showed low points in December, January, February, and March, followed by a rapid rise to a mode in July for those born alive and to a mode in June for those raised. Particularly large percentages of those born in May, June, and July were raised.

The percentage of males, though the differences are small, shows a high point in January followed by a gradual drop to July and August, except for the low point in April. The percentage of males rises to another high point in September, dropping again to a low point in November. There is some indication of a reciprocal relation of this curve to some of the other indices.

## DISCUSSION

Seasonal variation in the breeding of wild animals is common. Under domestication it appears that most classes of livestock breed throughout the year according to the desires of the stockman as to when he wants the young to come, based on likely market demand and when the young can be cared for best. Little consideration is given to the relative number of young, the mortality, or the birth weights and rate of growth of young born in the different seasons of the year.

From the data presented, the summer months, June, July, and August, appear to be on an average the most favorable for frequency of litter, size of litter, and percentage of guinea pigs born alive. The percentage raised of those born alive in these months is also high, but for this group the high period begins for those born in May. Definite peaks in the birth weight and gain curves point to May as the optimum month for birth. Most of the indices are generally high during the summer and early fall; birth weights show a second mode for guinea pigs born in October.

The months of December, January, February, and March were, on an average, generally the most unfavorable for the expression of the indices, a single high point in the frequency of litters in March being the exception.

Since the expressions of the various indices are highest during the summer the favorable environmental conditions that caused such expression, particularly in frequency and size of litter, must have occurred from two to three months earlier; that is, in the spring, probably in the early spring. This assumes that the reaction in frequency of litter is almost immediate, while the number of ova produced, which is the main factor in size of litter, requires a few days longer to be affected. The expression of birth weights is somewhat earlier but gives ample time for the dam to be influenced and still transfer the effect to the young in utero during the last half of the gestation period, this being the time when the major part of the growth of the fetus occurs and when the variations in the birth weights would most likely result. Gains appear to be more closely associated with the birth weights of the individual than with temporary environment as shown by the parallelism of these two curves. Percentage raised either comes closest to being an expression of conditions after birth as it is among the first to show a sharp rise, or it is affected by conditions quite different from those that influence the other indices.

In considering the monthly variations one should not overlook the fact that the basic data for the calculations are taken from the records of several different families, which no doubt react differently to a specific influence. Further, the data for the combined months can not be thought of as representative of any particular year but may be taken as an average expression of conditions in the seasons for the 18-year period. Variations in temperature, sunshine, rainfall, or other meteorological conditions within and without the heated colony house cause extreme variations from the normal, which may not be repeated in successive years, but it is felt that fairly accurate factors for eliminating the effect of season may be obtained from the combined data for general purposes or for those records for particular months of particular years as may be desired.



The discussion of the seasonal variations in the indices would not be complete without reference to the sex ratio. The variations in the individual months are so irregular that they are related to the other indices with some difficulty, but in the combined months the percentage of males is high in January, February, and March, when other indices are low, and high in September and October, when the indices for mortality and gains are dropping. This would point toward a higher prenatal mortality of females when conditions are poor, which is not in conformity with the general belief of greater prenatal mortality of males. With the large amount of data on which this study is based and the fact that many of the differences, particularly the larger ones, are more than three times the probable error, it seems proper to conclude that there were significant seasonal variations in the sex ratio in the guinea-pig data studied.

#### CORRELATIONS OF MONTHLY INDICES

The primary object in determining the monthly indices was for calculating the relation between the various measurable components of fertility and related factors over several months. Simple correlations were calculated between the eight monthly indices within the same month and for each one with each of the others for the four preceding and four succeeding months, using as a basis the monthly indices from December, 1906, to December, 1924. The correlations thus indicated the relationship between the different indices over a 9-month period.

The correlation coefficients calculated in this way are presented in Table 10 and in Figures 11 to 16. The month designated as 0 refers to the basic month for the group. For example, the correlations of litters per 100 matings with the other indices are given for litters per 100 matings born in the 0 month as related to itself, size of litter, etc., in each of the other months. Those at the left of the 0 month, designated by minus signs, refer to months preceding, and those at the right, designated by plus signs, refer to months succeeding the 0 month.

The index to which each curve in the figures applies is indicated on the margin. The distance between the horizontal lines represents values from 0 to 1.00 on the correlation scale.

As was previously explained, the indices for gains and percentage raised of guinea pigs born alive were recorded for the month in which the guinea pigs were born but were really expressed 33 days later. For this reason in the plotting of the correlation coefficients these indices have been advanced 33 days in their relation to the other indices, and vice versa. In Table 10 they have been advanced one month to show more clearly the relation between the expression of each index. Net fertility was calculated for the guinea pigs born in a certain month and therefore applies to a combination of conditions before birth and 33 days after birth. It, therefore, does not express conditions at birth nor conditions during the succeeding month. Its relation to the other indices is presented, however, as it is the net measure of fertility, being the number of young raised from those born in a particular month per 100 matings in that month. Any other means of calculating an index of this sort appeared to be subject to similar or greater criticism.

TABLE 10.—Correlation coefficients between the various indices

[Values in the 0 month as correlated with the various monthly indices over a 9-month period]

## LITTERS PER 100 MATINGS

Index	Correlation coefficient when the preceding or succeeding month is—										
	—5	—4	—3	—2	—1	0	+1	+2	+3	+4	+5
Litters per 100 matings.....	-----	+0.029	+0.133	+0.120	-0.042	+1.000	-0.042	+0.120	+0.133	+0.029	-----
Size of litter.....	-----	+0.060	+0.152	+0.087	+0.207	+0.460	+0.301	+0.259	+0.114	+0.142	-----
Birth weights of those raised.....	-----	-0.003	+0.009	+0.165	+0.220	+0.204	+0.059	+0.070	+0.137	+0.015	-----
Gain to weaning.....	-----	-----	+0.008	+0.205	+0.369	+0.274	+0.206	+0.123	+0.050	+0.061	-0.070
Percentage born alive.....	-----	+0.070	+0.135	+0.156	+0.397	+0.298	+0.198	+0.051	+0.065	-0.014	-----
Percentage raised of those born alive.....	-----	-----	+0.101	+0.128	+0.345	+0.384	+0.195	+0.133	+0.074	-0.002	-0.045
Net fertility.....	-----	+0.117	+0.179	+0.259	+0.268	+0.718	+0.173	+0.192	+0.166	+0.034	-----
Percentage males.....	-----	+0.107	-0.196	-0.017	-0.130	-0.048	-0.128	-0.032	-0.008	+0.054	-----
SIZE OF LITTER											
Litters per 100 matings.....	-----	+0.142	+0.114	+0.259	+0.301	+0.460	+0.207	+0.087	+0.152	+0.060	-----
Size of litter.....	-----	+0.301	+0.293	+0.429	+0.563	+1.000	+0.563	+0.429	+0.293	+0.301	-----
Birth weights of those raised.....	-----	+0.290	+0.429	+0.547	+0.552	+0.417	+0.352	+0.347	+0.296	+0.189	-----
Gain to weaning.....	-----	-----	+0.443	+0.628	+0.690	+0.554	+0.397	+0.278	+0.197	+0.075	+0.086
Percentage born alive.....	-----	+0.241	+0.345	+0.543	+0.512	+0.470	+0.241	+0.129	+0.024	+0.068	-----
Percentage raised of those born alive.....	-----	-----	+0.345	+0.488	+0.591	+0.594	+0.428	+0.315	+0.185	+0.095	+0.014
Net fertility.....	-----	+0.355	+0.415	+0.611	+0.658	+0.743	+0.440	+0.271	+0.217	+0.155	-----
Percentage males.....	-----	-0.073	-0.124	-0.159	-0.082	-0.113	-0.020	-0.019	-0.009	-0.069	-----
BIRTH WEIGHTS OF THOSE RAISED											
Litters per 100 matings.....	-----	+0.015	+0.137	+0.070	+0.059	+0.204	+0.220	+0.165	+0.009	-0.003	-----
Size of litter.....	-----	+0.189	+0.296	+0.347	+0.352	+0.417	+0.552	+0.547	+0.429	+0.290	-----
Birth weights of those raised.....	-----	+0.468	+0.534	+0.606	+0.753	+1.000	+0.753	+0.606	+0.534	+0.468	-----
Gain to weaning.....	-----	-----	+0.460	+0.504	+0.605	+0.747	+0.701	+0.510	+0.381	+0.305	+0.217
Percentage born alive.....	-----	+0.218	+0.302	+0.285	+0.323	+0.435	+0.417	+0.213	+0.101	+0.057	-----
Percentage raised of born alive.....	-----	-----	+0.319	+0.319	+0.409	+0.500	+0.538	+0.462	+0.306	+0.176	+0.153
Net fertility.....	-----	+0.246	+0.326	+0.314	+0.388	+0.518	+0.509	+0.401	+0.217	+0.146	-----
Percentage males.....	-----	+0.008	-0.063	-0.083	-0.094	-0.119	-0.118	-0.016	-0.009	+0.023	-----
GAINS TO WEANING											
Litters per 100 matings.....	-0.070	+0.061	+0.050	+0.123	+0.205	+0.274	+0.369	+0.205	+0.008	-----	-----
Size of litter.....	+0.036	+0.075	+0.197	+0.273	+0.397	+0.554	+0.690	+0.628	+0.443	-----	-----
Birth weights of those raised.....	+0.217	+0.305	+0.381	+0.510	+0.701	+0.747	+0.605	+0.504	+0.460	-----	-----
Gain to weaning.....	-----	+0.254	+0.364	+0.524	+0.757	+1.000	+0.757	+0.524	+0.364	+0.254	-----
Percentage born alive.....	+0.113	+0.223	+0.344	+0.412	+0.551	+0.618	+0.504	+0.279	+0.131	-----	-----
Percentage raised of those born alive.....	-----	+0.153	+0.223	+0.361	+0.537	+0.694	+0.695	+0.515	+0.332	+0.209	-----
Net fertility.....	+0.061	+0.189	+0.276	+0.439	+0.601	+0.694	+0.669	+0.470	+0.235	-----	-----
Percentage males.....	+0.101	-0.003	-0.012	-0.103	-0.162	-0.121	-0.108	-0.064	+0.017	-----	-----
PERCENTAGE BORN ALIVE											
Litters per 100 matings.....	-----	-0.014	+0.065	+0.051	+0.198	+0.298	+0.397	+0.156	+0.135	+0.070	-----
Size of litter.....	-----	+0.068	+0.024	+0.129	+0.241	+0.470	+0.512	+0.543	+0.345	+0.241	-----
Birth weights of those raised.....	-----	+0.057	+0.101	+0.213	+0.417	+0.435	+0.323	+0.285	+0.302	+0.218	-----
Gain to weaning.....	-----	-----	+0.131	+0.279	+0.504	+0.618	+0.551	+0.412	+0.344	+0.223	+0.113
Percentage born alive.....	-----	+0.055	+0.086	+0.300	+0.476	+1.000	+0.476	+0.300	+0.086	+0.055	-----
Percentage raised of those born alive.....	-----	-----	+0.062	+0.192	+0.447	+0.595	+0.643	+0.454	+0.306	+0.195	+0.124
Net fertility.....	-----	+0.050	+0.138	+0.301	+0.509	+0.702	+0.583	+0.418	+0.289	+0.184	-----
Percentage males.....	-----	+0.056	+0.054	-0.018	-0.118	-0.230	-0.044	-0.012	+0.023	+0.061	-----

TABLE 10.—Correlation coefficients between the various indices—Continued

## PERCENTAGE RAISED OF THOSE BORN ALIVE

Index	Correlation coefficient when the preceding or succeeding month is—										
	−5	−4	−3	−2	−1	0	+1	+2	+3	+4	+5
Litters per 100 matings.	−0.045	−0.002	+0.074	+0.133	+0.195	+0.384	+0.345	+0.128	+0.101	-----	-----
Size of litter.	+0.014	+0.095	+0.185	+0.315	+0.428	+0.594	+0.591	+0.488	+0.345	-----	-----
Birth weights of those raised.	+0.153	+0.176	+0.306	+0.462	+0.538	+0.500	+0.409	+0.319	+0.319	-----	-----
Gain to weaning.	-----	+0.209	+0.332	+0.515	+0.695	+0.694	+0.537	+0.361	+0.223	+0.153	-----
Percentage born alive.	+0.124	+0.195	+0.306	+0.454	+0.643	+0.595	+0.447	+0.192	+0.062	-----	-----
Percentage raised of those born alive.	-----	+0.117	+0.223	+0.417	+0.727	+1.000	+0.727	+0.417	+0.223	+0.117	-----
Net fertility.	+0.061	+0.159	+0.312	+0.511	+0.706	+0.724	+0.562	+0.328	+0.211	-----	-----
Percentage males.	+0.126	+0.074	−0.045	−0.134	−0.118	−0.102	−0.075	−0.028	+0.044	-----	-----
NET FERTILITY											
Litters per 100 matings.	-----	+0.034	+0.166	+0.192	+0.173	+0.718	+0.268	+0.259	+0.179	+0.117	-----
Size of litter.	-----	+0.155	+0.217	+0.271	+0.440	+0.743	+0.658	+0.611	+0.415	+0.355	-----
Birth weights of those raised.	-----	+0.146	+0.217	+0.401	+0.509	+0.518	+0.388	+0.314	+0.326	+0.246	-----
Gain to weaning.	-----	-----	+0.235	+0.470	+0.669	+0.684	+0.601	+0.439	+0.276	+0.189	+0.061
Percentage born alive.	-----	+0.184	+0.289	+0.418	+0.583	+0.702	+0.509	+0.301	+0.138	+0.050	-----
Percentage raised of those born alive.	-----	-----	+0.211	+0.328	+0.562	+0.724	+0.706	+0.511	+0.312	+0.159	+0.061
Net fertility.	-----	+0.203	+0.337	+0.486	+0.616	+1.000	+0.616	+0.486	+0.337	+0.203	-----
Percentage males.	-----	+0.074	−0.098	−0.062	−0.155	−0.167	−0.102	−0.040	+0.008	+0.015	-----
PERCENTAGE MALES											
Litters per 100 matings.	-----	+0.054	−0.008	−0.032	−0.128	−0.048	−0.130	−0.017	−0.196	+0.107	-----
Size of litter.	-----	−0.069	+0.009	−0.019	−0.020	−0.113	−0.082	−0.159	−0.124	−0.073	-----
Birth weights of those raised.	-----	+0.023	−0.009	−0.016	−0.118	−0.119	−0.094	−0.083	−0.063	+0.008	-----
Gain to weaning.	-----	-----	+0.017	−0.064	−0.108	−0.121	−0.162	−0.103	−0.012	−0.003	+0.101
Percentage born alive.	-----	+0.061	+0.023	−0.012	−0.044	−0.230	−0.118	−0.018	+0.054	+0.056	-----
Percentage raised of those born alive.	-----	-----	+0.044	−0.022	−0.075	−0.102	−0.118	−0.134	−0.045	+0.074	+0.126
Net fertility.	-----	+0.015	+0.008	−0.040	−0.102	−0.167	−0.155	−0.062	−0.098	+0.074	-----
Percentage males.	-----	+0.076	−0.102	+0.008	+0.047	+1.000	+0.047	+0.008	−0.102	+0.076	-----

In the presentation of correlation coefficients the probable errors are not given, but they are easily estimated. The correlations are based on 213 months' records in all cases, the first four months' indices which were calculated from smaller numbers of individuals only being used in the correlations with indices in months preceding and succeeding the 0 months. The formula for the probable error

for the simple correlation coefficient is  $0.6745 \frac{1-r^2}{\sqrt{n-2}}$ , where  $r$  is the

correlation coefficient and  $n$  is the number of pairs correlated. The probable error of any coefficient may thus be determined by multiplying  $1-r^2$  by 0.046. The probable error is 0.046 for correlations from 0.00 to 0.10 and is reduced for larger coefficients.

It will be noted that in general the nine correlations between the indices of a particular character and those for indices of another character in nine successive months form curves starting with lower values and gradually rising to higher values, dropping off toward the end to lower values. For the purpose of final analysis it was desired to know the point at which the maximum relation between different

indices occurs, which makes necessary an estimation of the mode of each curve of the correlation coefficients. A thoroughly satisfactory method of estimating the true course of the curves was not evident.



FIGURE 11.—Correlations between litters per 100 matings and the other indices in successive months, designated -4 to +5. The 0 months refer to happenings in the same month, while the correlations in -1, -2, -3, etc., 7 months are for the correlations between happenings in the first, second, and third preceding months, and frequency of litter, in the 0 month. Likewise, the correlations between happenings in the first, second, third, etc., succeeding months and the frequency of litter in the 0 month are indicated by +1, +2, +3, etc.

One method considered was to fit some mathematical formula to each, but the question arose as to which points at the ends of each curve should be omitted and what equation should be used that would obviously be different for each curve. The choice of such a method

is purely arbitrary, and the measure of success is the judgment of the eye. It therefore seemed as well to attempt to fit by the eye in the first place, when so much irregularity existed.

The correlations are briefly discussed below, first with reference to the more significant ones and finally with reference to the relationship

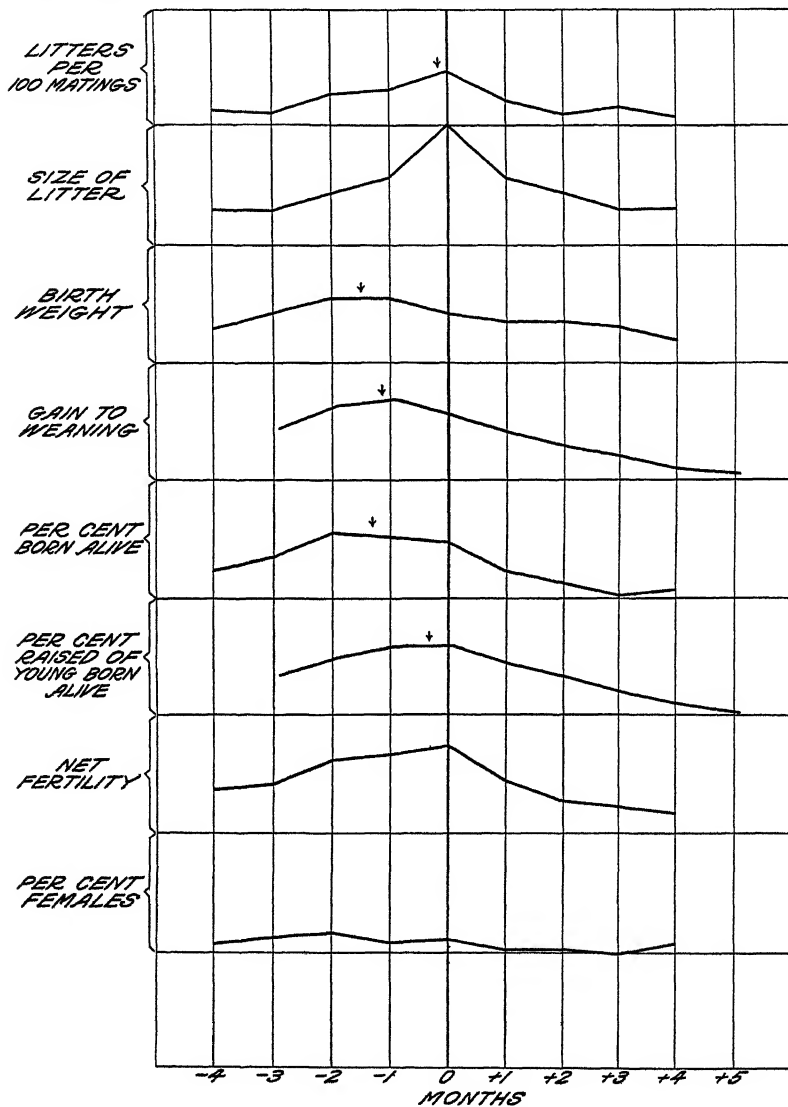


FIGURE 12.—Correlations between size of litter and other indices in successive months. Explanation as of Figure 11

and time interval between expressions of maximum relationship from which estimates of the major influences of common conditions on the different indices were made. The interpretation of the correlations must be made on the basis of related reaction to simultaneous or related conditions and not on a cause-and-effect basis, since there is no apparent reason for such a relationship in most cases.

## LITTERS PER 100 MATINGS

The correlations between litters per 100 matings and the other indices were not entirely satisfactory because of the irregular behavior of this index from month to month. This irregularity resulted in generally

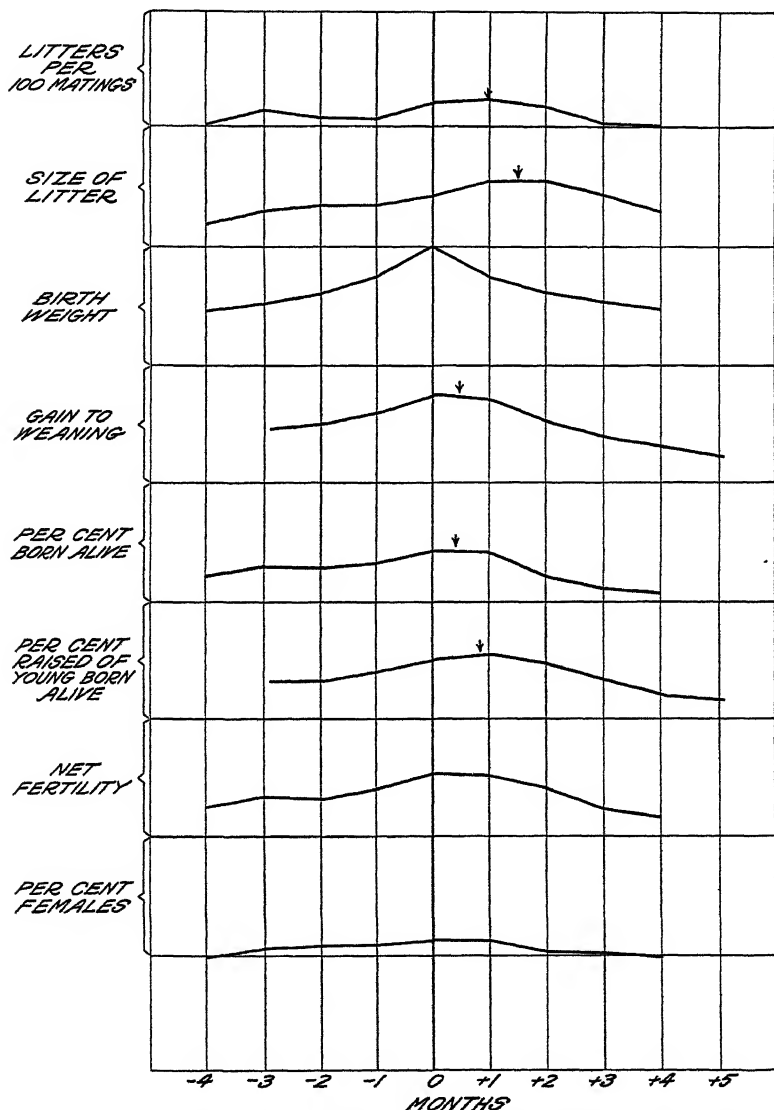


FIGURE 13.—Correlations between birth weights and other indices in successive months. Explanation as of Figure 11

low correlations, the highest being 0.46 in the 0 months, with size of litter, followed closely by 0.40 for percentage of young born alive in the -1 month and 0.38 for percentage raised in the 0 month.

It is quite obvious that both frequency and size of litter are largely determined at the time of conception, but the influences must also be

operative before conception, and both may be modified during gestation, frequency of litter by abortions and complete resorption of litters, and size of litter by resorption of individuals. The same environmental effects may, however, not operate at the same time on both

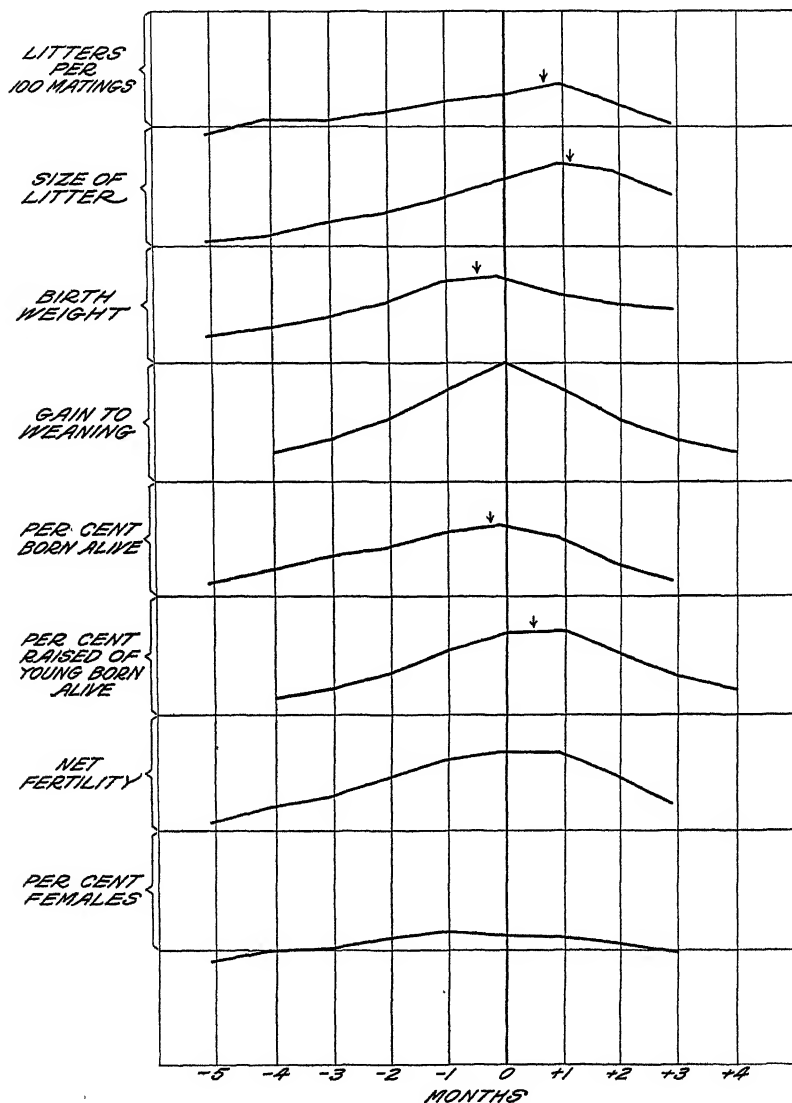


FIGURE 14.—Correlations between gains and other indices in successive months. Explanation as of Figure 11

indices and the two may respond differently. There is some indication of difference in response, as the curve of the correlation coefficients between the two is maintained higher for the following (+) months than for preceding (-) months. This departure from symmetry appears to be about one month. This thus indicates that size

of litter, while evidencing the maximum effect of a particular influence in the same month as frequency of litter, is somewhat slower in its reaction, showing more lag in its general response. The lag of the maximum correlation between frequency and size of litter was estimated from the mode of the correlation curve at +5 days. Assuming

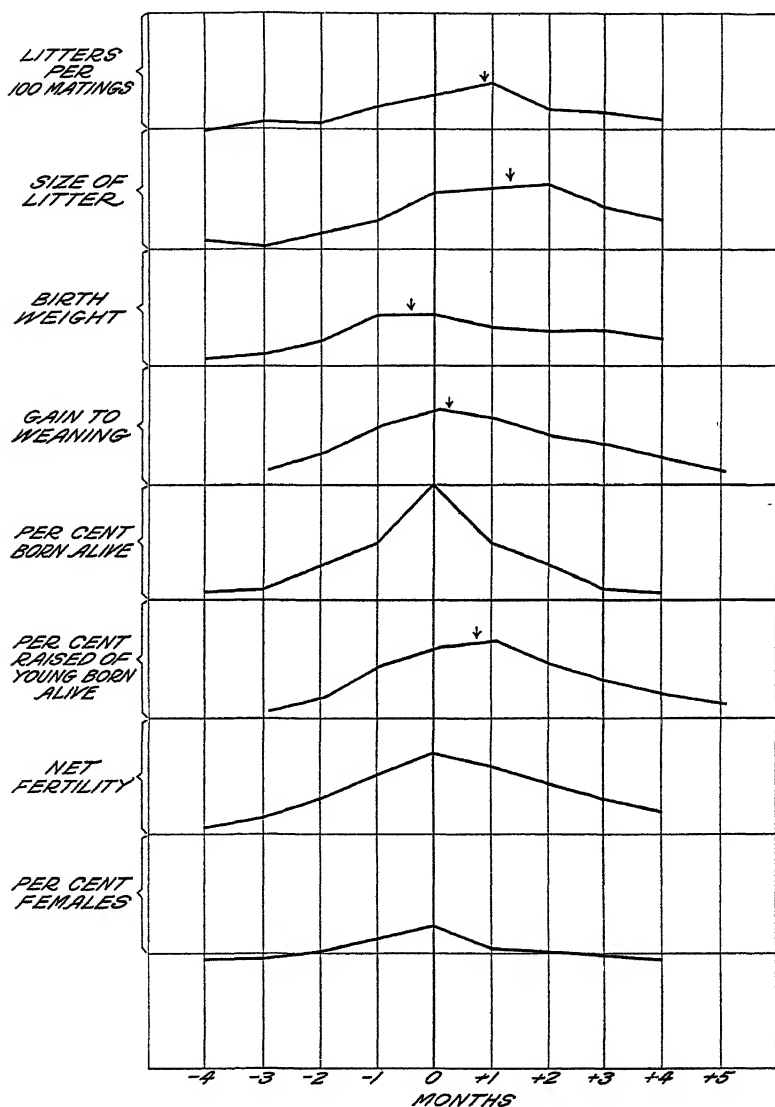


FIGURE 15.—Correlations between percentage born alive and other indices, in successive months. Explanation as of Figure 11

that the latest that frequency of litter could be influenced would be at conception, size of litter, being five days slower in responding, would be affected in litters conceived five days later. Thus conditions associated with frequency at conception are associated with the number of ova produced by females conceiving five days later.



As one would expect, the percentage of young born alive reacts somewhat more promptly to a common influence than frequency of litter. It is readily understood how an extremely unfavorable environmental condition, such as extreme cold, acting at birth, might

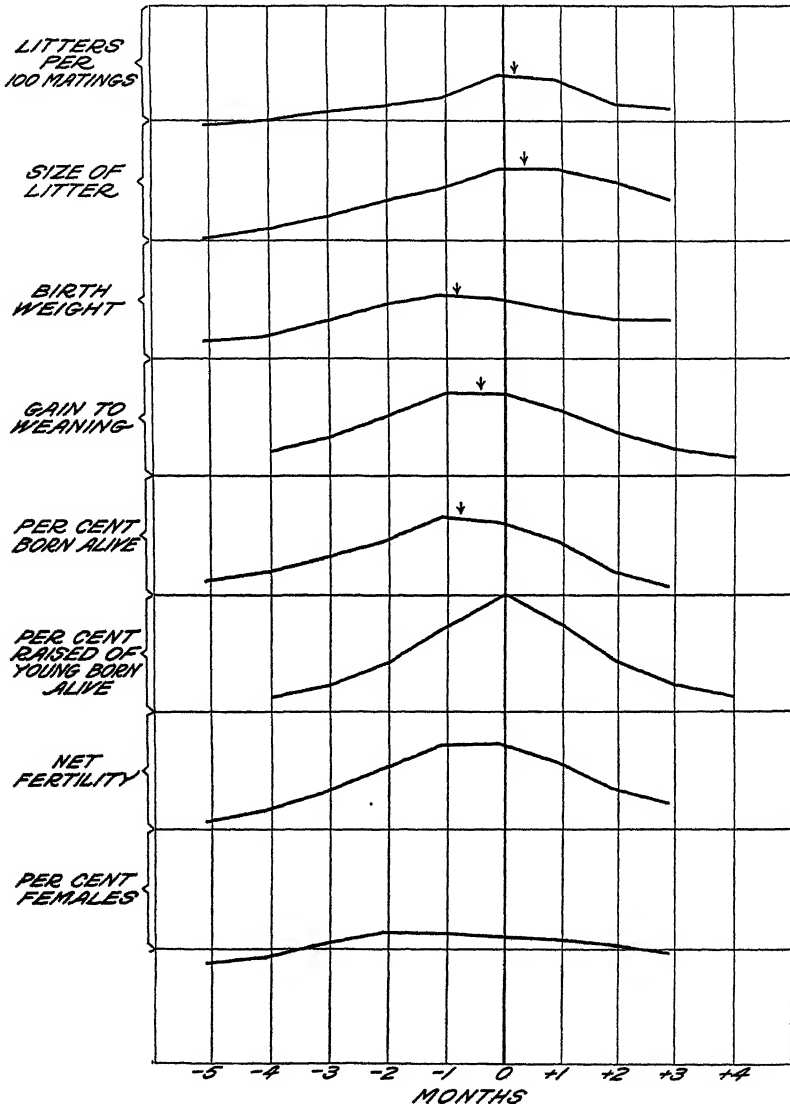


FIGURE 16.—Correlations between percentage raised of guinea pigs born alive and other indices in successive months. Explanation as of Figure 11

destroy entire litters. However, as indicated by the correlation coefficients, the reaction to such immediate conditions is not of primary importance, or the time relations would be more widely separated than one month. Three of the five significant correlations of frequency of litter and percentage born alive are with percentage

born alive before the 0 month, with the estimated mode of the maximum relation 26 days before the time of expression of frequency of litter. In calculating back it is found that the young expressing the maximum relation of percentage born alive to litters per 100 matings were at the twenty-sixth day of the gestation period<sup>5</sup> when the litters by which frequency was measured were conceived. Thus, it may be assumed that the maximum concomitant influences on frequency of litter and percentage born alive occurred, respectively, at conception and at the twenty-sixth day of gestation. The course of the curve showing more lag in the 0 and + months than in the - months indicates that the influence on percentage born alive is greater prior to the twenty-sixth day of gestation than after that point is reached.

The mode of the correlation curve between frequency of litter and percentage of young raised of those born alive was estimated at -5 days. The percentage raised is expressed 33 days after birth; therefore, the guinea pigs expressing percentage raised are born 38 days before those expressing frequency of litter. Therefore, at the time those expressing frequency of litter are conceived (-68 days) those expressing percentage raised are at the thirty-eighth day of the gestation period.

The correlations of frequency of litter with birth weights and gains were relatively low. It is, however, apparent that the mode for the relation of frequency of litter to the weights and gains occur somewhere during the -1 month, and the points were estimated for gains at -20 days and for birth weights at -29 days. As gains were expressed at 33 days of age, the young which showed the effect of gain 20 days before the effect on frequency was apparent were 33 days old. The influence must have operated 68 days before the litters expressing frequency were born. It therefore acted on gain  $68 - (20 + 33) = 15$  days before birth, or at the fifty-third day of gestation. Similarly, from the concomitant variations between frequency of litter and birth weights the influence acted on birth weights at the twenty-ninth day of gestation.

#### SIZE OF LITTER

Size of litter, unlike frequency of litter, shows a relatively high correlation with itself in successive months, and with the other indices except frequency of litter. (Table 10 and fig. 12.) It not only maintains a high correlation between the first and second succeeding months, +0.56 and +0.43, but for three and four months away the correlation is practically +0.30. This seems to indicate that size of litter is the result of an accumulation of environmental effects or that the environmental factors affecting size of litter are associated.

Because of the delayed evidence of the size of litter—at the end of the 68-day gestation period—most of the higher correlations were in either the 0 or - months. The highest correlations were +0.69 and +0.63, with gains in the -1 and -2 months; +0.59 with percentage raised in each of the 0 and -1 months; +0.54 and +0.51 with percentage born alive in the -2 and -1 months; and +0.55 with birth weights in each of the -1 and -2 months, respectively.

The 9-month period for which the correlations were calculated was not sufficient to bring out all the existing relationships between size of litter and the other indices, particularly those for birth weight,

<sup>5</sup> The gestation period varies from 65 to 71 days, but for convenience it has been here considered as 68 days.

gains, and percentage of young raised of those born alive. Likewise, there were correlations of  $+0.30$  between the average size of the litters born six months apart.

The correlation curves for size of litter were smoothed and the modes estimated, as shown in Figure 12, in the same manner as for the frequency-of-litter correlations. From the modes of these curves, it was estimated that variations in the other indices, showing the maximum degree of association with variations in size of litter, were expressed the following number of days prior to the birth of the litter from which size of litter was measured: Frequency of litter, 5 days; birth weight, 45; gain, 34; percentage of young born alive, 39; and percentage raised, 10 days. If it requires five days before conception for an influence to act on size of litter (see p. 151), the common influence on size of litter and the other indices may be calculated to have their maximum effect on frequency of litter at conception, and on the other indices at the following stages of gestation: Percentage born alive, 34 days; birth weights, 40; percentage of young raised, 38; and gains, 62 days.

#### WEIGHTS

Birth weights correlated with birth weights and gains correlated with gains over the 9-month period present similar curves, except that the relationship between birth weights evidently extends over a longer time. The correlation for birth weights separated over a period of four months is  $+0.47$ , while the correlation for gains over a similar period is  $+0.25$ . The correlation between birth weights in the succeeding months was  $+0.75$  and between gains in succeeding months  $+0.76$ . The correlations between birth weight and gain are relatively very high. The correlation between the birth weights and the gains made by the same individuals,  $+0.70$ , was not quite so large as the correlation between the birth weights and gains made at the same time,  $+0.75$ , by different individuals.

There were relatively high correlations of both birth weights and gains with the other indices in several months, as shown in Table 10 and Figures 13 and 14. There was no index, except litters per 100 matings, which did not in some months show a correlation above  $+0.40$  with birth weight and gain.

Several of the birth-weight-correlation curves showed a bimodal tendency, or at least were very flat. This was particularly pronounced for the correlations with percentage of young born alive, frequency of litter, and size of litter, and there appeared to be some indication of such a relationship with percentage raised and gain. This may be explained as due to the bimodal characteristic of the birth-weight index noted when the seasonal variations in the combined months were discussed.

The gain correlation curves were somewhat steeper than those for birth weight, otherwise the relationships were similar, though gains in successive months were not quite so closely correlated.

The modal relation between the manifestation of characteristics showing maximum relations were, with birth weights as a base, at +29 days for frequency of litter, +45 days for size of litter, +14 days for gains, +12 days for percentage of young born alive, and +25 days for percentage raised. The modes of the gain correlation curves were established at +20 days for frequency of litter, +34 days for size of litter, -14 days for birth weight, -8 days for percentage of young

born alive, and +13 days for percentage raised. The modal relation between birth weights and the other indices thus occurs from 9 to 20 days later than the modal relation between gains and the same indices.

The weight curves bring out the similarity of the reaction of intrauterine and extrauterine life to the environment, but the birth-weight and gain relationships with the other indices are distinctive in the relatively greater tendency for the birth weights to be affected over a longer period. A comparison of the correlations between indices expressed at birth with those expressed at weaning shows that there is generally greater correlation between expressions in the same month than between the indices for the same individuals expressed in successive months.

#### MORTALITY

In contrast with most of the curves for the correlations with birth weights, the 9-month period covers practically the entire time over which there are significant relationships for percentage of young born alive and percentage raised of those born alive, both with themselves and with other indices. (Table 10 and figs. 15 and 16.) The curve that shows percentage born alive correlated with itself is particularly steep, and the significant relations cover five months, i. e., two months in either direction from the 0 month. The significant relations between the percentage raised of those born alive in successive months covers about seven months, but the relation is dwindling out pretty rapidly two months away. The curves for the correlation of percentage born alive and percentage raised with the other characteristics are similar in shape, and the values are relatively uniform, except that those for percentage raised are somewhat higher and the curves are broader. The highest correlation between the mortality percentages was 0.64 for the same litters; i. e., percentage born alive in the 0 month and percentage raised in the +1 month. Evidently, if conditions were favorable for low mortality at birth, a relatively large percentage survived to weaning.

Unlike the weight correlation curves, there were several indications of closer relationship between the monthly indices for the same individuals expressed at different times than for those of different individuals expressed at the same time.

It is curious that percentage of young born alive was not so closely correlated with itself in succeeding months as it was with certain of the other indices in those months. There were significantly higher correlations for four months with percentage raised, three months with gains, and two months with size of litter. Excluding litters per 100 matings, there were correlations above +0.5 in two or more months between the mortality percentages and all the other indices, except for the relationship between birth weight and percentage of young born alive, for which there were two months above +0.4.

The correlations with percentage of young born alive formed sharper peaked curves than the correlations with percentage raised, though the correlations with the latter in the months of maximum relation were frequently a little larger. The difference between the expression of the modes of the percentage born alive and percentage raised curves when related to each other, was estimated at 23 days. If all of the indices were associated with the expression of the same factors, similar differences would be expected between the mode of

the correlation curves for (1) percentage born alive-frequency of litter and percentage raised-frequency of litter curves, and (2) percentage born alive-size of litter and percentage raised-size of litter curves, etc. This is approximated. The modes for the different index correlation curves with percentage born alive and percentage raised were, respectively: Frequency of litter, +26 and +5 days; size of litter, +39 and +10 days; birth weights, -12 and -25 days; and gains, +8 and -13 days. The differences thus range from 13 to 29 days.

#### NET FERTILITY

Net fertility is very closely related, mathematically, to litters per 100 matings, size of litter, percentage of young born alive, and percentage raised of those born alive; and the correlation coefficients calculated were +0.70 or more in all four cases, in the same month. (Table 10.) It is, however, interesting to note that there are other correlations nearly as great in other months. For example, the coefficient of correlation with percentage raised in the +1 month, +0.71, is not significantly different from the correlation with the data forming a direct component of it. The correlations of net fertility with gains made during the month that the young are raised and during the gestation period, particularly during the last month of gestation, are nearly as large as the correlation between net fertility and other indices forming component parts of it. The correlations with the size of litter for the first two months after the young are born are +0.60 and +0.61. In fact, frequency of litter is the only one of the fertility or weight factors which does not show a relatively high correlation for several months before and after the 0 month.

#### SEX RATIO

Attention has already been called to the fluctuations in the sex ratio and the indication that these might be related to the seasons and possibly to variations in the other monthly indices. (Figs. 9 and 10.)

In the planning of monthly indices during the course of the study the sex ratio was tabulated as a matter of interest, but with the expectation that no correlation would be found with the other indices. However, among the correlations as a whole nearly all were positive, but of the 63 correlation coefficients between percentage of males and the other indices given in Table 10, there were only 17 which were positive, and all of these were with indices three months or more from the month in which the sex ratio was measured. Twelve were at the extreme end of the period and all were small. The largest positive correlations were +0.10 in the +5 month for gains, +0.13 in the same month for percentage raised, and +0.11 in the +4 month for frequency of litter. There is, at least, one month in which the correlation of the sex ratio with each of the other indices is -0.12 or larger. With litters per 100 matings the alternation of the months in the degree of relationship is quite distinct. The correlations of percentage of males in one month with frequency of litter in the next month was -0.13; and between the sex ratio in one month and frequency of litter in the third following month -0.20. The correlations of percentage of males in one month with size of litter in the same month and in the three succeeding months were -0.11, -0.08,

-0.16, and -0.12, respectively. The correlations with birth weights in the same and in the preceding months were both -0.12 and in the succeeding month -0.09. The relation to gains covers a similar period, except that the correlations were somewhat higher, being -0.11, -0.12, and -0.16, and, in the second month later, -0.10. There are no possible significant correlations with percentage of young born alive except in the same month, which is the highest of the coefficients in this group, -0.23, and in the next following month, -0.12. The three highest correlations with percentage raised are within the same month and during the first and second succeeding months, being -0.10, -0.12, and -0.13, respectively.

Very little would be thought of the small correlations of percentage of males with the other indices if they were not so uniform and all in the same direction, thus indicating that a lower percentage of males is associated with all other indices which are assumed to result from favorable conditions. Percentage of young born alive showed the largest correlation with sex ratio, which is hard to understand since sex was found to be unrelated to mortality at birth. In the different litter sizes the values for the percentage born alive alternated for the sex ratio, and the percentage of the two sexes born alive was practically the same.

Further study of the correlations indicated that the variations in the sex ratio were most closely associated with variations in the percentage of young born alive. As the correlations were all small and there was only about 6 per cent of the variability in the sex ratio associated with the variations in the other indices in the months showing the highest correlations, they do not warrant further discussion.

#### DISCUSSION

The correlations between the indices ranged in the modal months in most cases from 0.40 to 0.75, indicating from 16 to 56 per cent association in the variations. These are obviously high for biological data so pronouncedly influenced by environmental fluctuations, and particularly when there appears to be an almost complete lack of cause-effect relationship. The curves showed a dwindling of the degree of correlation as the time interval increased, though in some cases the 9-month period employed was clearly not sufficient to get away from a considerable degree of association.

In order to bring out more clearly the interval between the maximum degree of associated variation in the different indices Figure 17 was constructed, showing diagrammatically the interval between the reaction of each pair of indices to a common influence. The intervals in days between the evidence of the maximum relations are indicated. Straight lines show the shortest period between such expressions and angles show the longer intervals. For example, frequency of litter was manifested 5 days before the size of litter, and birth weight 29 days before frequency of litter, but birth weight was evidenced 45 days before size of litter instead of 34 days before it. This was, however, the greatest discrepancy, as the differences in other cases vary from 11 days to complete agreement. Dotted lines were used in the figure where the totals are the same as the sum of the component intervals. For example, frequency of litter was evidenced 5 days before size of litter, and percentage of young raised

5 days before frequency of litter. Since percentage raised was 10 days before size of litter, the last two were connected by a dotted line.

There was in general a close agreement among the different paths for measuring the time of evidencing frequency of litter, percentage raised, gains, and percentage of young born alive, and between frequency of litter, percentage raised, and birth weights, as well as between size of litter, frequency of litter, and percentage raised; and percentage raised, gains, and birth weights. From the fact that limited groups can be selected which show similar relations, and that there are few wide departures from the sum of any paths which may be followed, it is convenient to discuss the associations as though all indices reacted to the same complex of conditions. Such are probably closely associated conditions, but these would not be expected to be identical.

Another matter of interest is the order in which different indices evidence the effects of changed environmental conditions. The

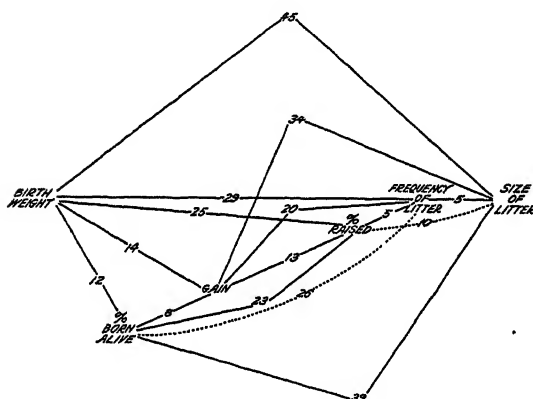


FIGURE 17.—Interval in days between maximum correlations between the expressions of the indices. For explanation see text

data throw light on this question only from the standpoint of associated variations in pairs of indices and do not contribute to the variations in a particular index resulting from a specific influence acting only on that index and having no influence on, or influencing to a minor degree, other indices studied. The duration of the intervals between the expression of the maximum amounts of concomitant variation in

the different indices permits the establishment of the order in which the reactions are expressed with considerable certainty. The order of a reaction to a particular stimulus is evidenced successively in (1) birth weights, (2) percentage of young born alive, (3) gains, (4) percentage raised, (5) frequency of litters, and (6) size of litter. This order is maintained for all the indices when considered with reference to any single one, but there is a slight overlapping when considered in relation to several. For example, variations in birth weights associated with frequency of litter are evidenced 29 days before frequency of litter, and size of litter 5 days after frequency of litter; but variations in percentage of young born alive associated with variations in size of litter are evidenced 39 days apart, placing the expression of percentage born alive before birth weight, when the latter is calculated from its relation to frequency of litter, and the former from its relation to size of litter. When birth weight is considered from its direct relation to size of litter it again evidences its variation associated with size of litter before the variations in percentage born alive are apparent.

Having established the relation between the time of expression of the associated variation in the different indices, it becomes of con-

siderable practical interest to know at what stage in the life history of the animal associated variations in the different indices are most likely to occur. The most promising lead to this is in a consideration of the relation of the various indices to size and frequency of litter. Obviously, these two indices must be mainly influenced before conception, but there appears to be no way of determining from the data just how long before conception. The fact that size of litter requires five days longer to react to certain conditions than frequency of litter helps, but it is not certain that frequency of litter responds immediately, nor is it to be expected that it should. For the want of a better starting point it may be reasoned that frequency of litter is affected at conception and size of litter five days before conception. McKenzie (9) found in swine particularly rapid growth of the follicles during prooestrus, with the elimination of some follicles at all times, which lends some support to the selection of such a base, and it is reasonable to believe that the elimination of follicles might be closely associated with environmental conditions.

Assuming that influences must act on size of litter five days before conception, we may estimate the time when the influences act on the other indices. The common influences between size of litter and birth weights would act on birth weights 28 days before birth, or at the fortieth day of the gestation period for birth weight. On the other hand, common influences between frequency of litter and birth weights act on birth weights at the twenty-ninth day of gestation. Similarly, common influences between frequency of litter and percentage of young born alive act on the latter at the twenty-sixth day of gestation, while with size of litter the action is placed at the thirty-fourth day of gestation. Common influences between frequency of litter and gain act on the latter at 15 days before birth, or at the fifty-third day of gestation, and when calculated from size of litter, at six days before birth or at the sixty-second day of gestation. The time of influence on percentage raised is placed at the thirty-eighth day of gestation from its relation to both size of litter and frequency of litters.

The other four indices may now be considered with reference to the time in the gestation period between the action on each. There is 25 days' difference in the expression of percentage of young raised and birth weights. Allowing for the expression of the former at weaning and the latter at birth, this makes percentage raised influenced  $33 - 25 = 8$  days later in the gestation period than birth weights. Similarly, gains are influenced 13 days later in the gestation period than percentage raised, 25 days later than percentage born alive, and 19 days later than birth weights. These intervals do not give any indication of the time in the life cycle of the animal or the duration of the time between the changes in the environment and the evidence of a reaction of a particular index to it. A threefold relation between two indices and the relation of the basic one to size of litters furnishes an indication of this, but offers some complications. For example, birth weight was estimated to be influenced at the fortieth day of gestation from its relation to size of litter, but at the twenty-ninth day of gestation from its relation to frequency of litter. When birth weight is used as the base calculated from its relation to size of litter, frequency of litter must be influenced 11 days after conception, which seems highly improbable. Similarly, when gain, percentage of young born alive, and percentage raised were employed



as the bases and when the influences acting on them were calculated from size of litter, frequency of litter was influenced 9, 8, and 0 days, respectively, after conception.

With a full appreciation of these small discrepancies, the most satisfactory way to get a clear interpretation of the meaning of the interval between the expression of the reaction of the different indices to common environmental conditions appeared to be by the selection of the most probable single interval between each index based on all the observations. The most probable interval between the expression of the different indices was calculated by the method of least squares, taking account of the 15 equations that could be set up from the intervals given in Figure 17.

The result of this calculation indicated that the different indices expressed their reaction to common influences as follows: First, birth weight, followed in order by percentage of young born alive, 7 days

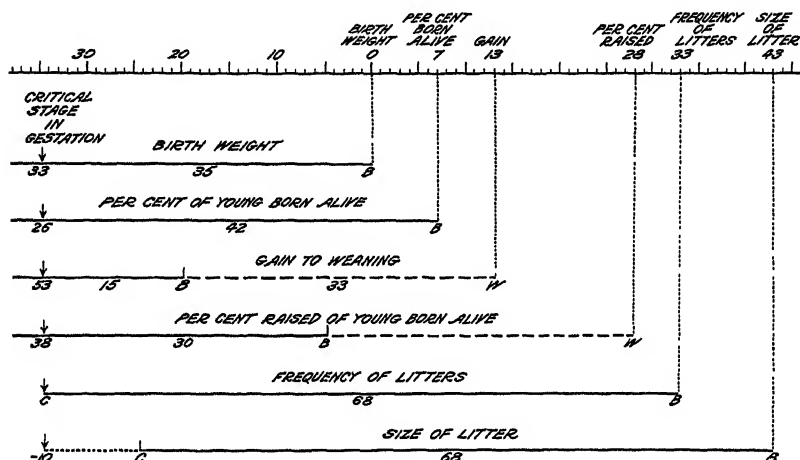


FIGURE 18.—Order of expression of maximum associated variation in the different indices and estimated critical stage in the development of each. The scale at the top represents the interval in days between the expression of the maximum correlations between the indices. In the lower portion of the figure the development of the individuals expressing each index is represented with the estimated critical stage in development for each. The letters C, B, and W refer to conception, birth, and weaning, respectively.

later; gain, 13 days later; percentage raised of those born alive, 28 days later; frequency of litter, 33 days later; and size of litter, 43 days after the litters expressing the associated variations in birth weights were born. These relationships are shown diagrammatically on the scale at the top of Figure 18.

It may be assumed that both size and frequency of litter are mainly influenced not later than conception. In the lower portion of Figure 18 the development of the individuals expressing different indices has been diagrammed. Birth weights, percentage of young born alive, frequency of litter, and size of litters are expressed at birth, while gain and percentage raised of those born alive are expressed at weaning, 33 days after birth.

From Figure 18 it is apparent that if the maximum influence of the common factors acts on frequency of litter at conception, the critical stage in development for the action on the other indices is 10 days before conception for size of litter and at the following stages in the

gestation period for the other indices: Birth weight thirty-third day, percentage of young born alive, twenty-sixth day, gain to weaning fifty-third day, and percentage raised thirty-eighth day of gestation.

On this basis it may also be estimated that the maximum variations in birth weight associated with the maximum variations in the other indices resulting from a common causal factor, may be expected to occur about 35 days after the operation of the causal factor. If this be correct, the time intervening between the occurrence of the common causal factor and the expression of its effect on the other indices may be estimated as follows: Percentage of young born alive, 42 days; gain to weaning, 48 days; percentage raised of those born alive, 63 days; frequency of litter, 68 days; and size of litter, 78 days.

The time of operation of a particular influence has been referred to as if it were specific, but the breadth of the correlation curves indicates that the influencing conditions extend over a considerable period and are more cumulative than immediate, and some more so than others. Judging from the curves, frequency of litters and percentage of young born alive are more affected by immediate conditions than the others, with size of litter showing the next greatest tendency in this direction. The specific time mentioned should be taken as the latest point in the life cycle at which the maximum influence occurs. It is probable that size of litter and frequency of litter react to influences earlier than those used as a basis. However, Loeb (8) showed that there were no large follicles in the guinea-pig ovary after oestrus, and therefore frequency and size of litter would be expected to be the result of conditions acting at some time during the 17-day period prior to the next ovulation. Papanicolaou (12) found that ovogenesis in the guinea pig was going on at all times, and that the rate was stimulated by favorable nutrition, seasonal conditions, and oestral activity, and retarded but not stopped by the presence of corpora lutea and competition in the ovary. These findings have a definite bearing on the estimate of the time at which the frequency and size of litters might be influenced, and indicate that the time selected is within the limits of expectation.

There is no doubt that it takes several days, if not weeks or months, in particular cases for conditions to affect a dam's physiological condition so that she will ovulate and conceive, whereas the time relations have been based on this as an immediate effect. Furthermore, size of litter is no doubt affected at least by the beginning of the cycle, culminating in ovulation and the conception of the litter. The constant elimination of follicles from the ovary throughout the various stages in development has been cited by several authors. It thus appears to be a cumulative process, though there may be critical periods with heavy elimination, or times beyond which further atresia is a minor matter. The operation of such influences during the preceding period with an ovulation period intervening would not seem unreasonable.

If seasonal variations in the sex ratio are admitted, of which there seems to be considerable evidence, these variations must be associated with conditions other than those causing the variations in the other indices, with the possible exception of percentage of young born alive; a sex-difference in the prenatal mortality at an early stage in gestation was the most plausible means by which the variations in the sex ratio could be brought about.

## SUMMARY AND CONCLUSIONS

From a study of the fertility, mortality, birth and weaning weights, and sex ratio in an inbred guinea-pig colony comprising more than 30,000 individuals born during a period of 18 years, the following results were obtained and conclusions reached:

1. Size of litter affected birth weights, weaning weights, and gains to weaning; the weights were larger in all cases for guinea pigs born in the small litters. The reciprocals of the weights were fitted closely by a second-order parabola.

2. Size of litter affected the percentages of guinea pigs born alive and the percentages raised of those born alive; litters of two and three were most favorable but the mortality was considerably greater in the larger litters.

3. Size of litter did not affect the sex ratio of the total young born.

4. The birth weights of those which were raised to weaning age were about 10 gm. heavier than for those which died between birth and weaning. The birth weights of those born dead were approximately 4.5 gm. lighter than those dying between birth and weaning. These differences were maintained constantly throughout the different-sized litters and were statistically significant.

5. Sex was a small but constant factor in influencing birth weights, weaning weights, and gains; males were heavier than females in all litter sizes. The birth weights were similarly affected by sex for those which died between birth and weaning and those raised to weaning, but the difference in those born dead was not significant.

6. The percentage of young born alive was not affected by sex, but over 1 per cent more males than females were raised to weaning of the guinea pigs born alive. This relation was relatively uniform in all litter sizes.

7. Correction factors were developed for eliminating the effect of sex and litter size on the birth weights of guinea pigs born dead, those that died, and those that were raised. Similar correction factors were developed for weaning weights and percentage raised. Correction factors for the effect of size of litter on percentage of young born alive were likewise developed.

8. Monthly indices were calculated, for 217 months, for frequency of litter, size of litter, birth weights, gains to weaning, percentage of young born alive, percentage raised of those born alive, number of young raised per 100 matings, and sex ratio, after making suitable corrections for the effect of size of litter and sex of young.

9. Most of the characteristics showed definite seasonal cycles, conditions being generally unfavorable in the late fall and winter and favorable in the spring and summer. Birth weights showed a bimodal seasonal cycle with higher birth weights in the late spring and fall. There were other exceptions in individual characteristics and for particular years. The sex ratio also showed some seasonal variations.

10. From the simple correlations of each of these characteristics with each other over a 9-month period it was apparent that:

- (a) Frequency of litter showed the lowest correlation with itself and with each of the other factors, followed in order by size of litter, indicating variations associated with more immediate conditions. This is also true to a less extent of percentage of young born alive. On the other hand, birth weights, gains, and percentage raised of those born alive appeared to result from influences operating over a longer time.

(b) It was also apparent that the different indices were probably affected by different influences, but for simplification of explanation the approximate time in the life history of the animal before which the indices are most likely to be affected was calculated from the composite relations as 10 days before conception for size of litter, conception for frequency of litter, twenty-sixth day of gestation for percentage of young born alive, thirty-third day of gestation for birth weight, thirty-eighth day of gestation for percentage raised of those born alive, and fifty-third day of gestation for gain. It was estimated that the reaction to exceptional conditions would be expected to manifest itself first in birth weight, followed in 7 days by percentage of young born alive, in 13 days by gain, in 28 days by percentage raised of those born alive, in 33 days by frequency of litter, and in 43 days by size of litter.

11. From the correlations a small but regular negative association appeared between the percentage of males on the one hand and all the other indices indicating favorable conditions on the other hand.

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# THE EFFECT OF ETHYLENE ON THE CHEMICAL COMPOSITION AND THE RESPIRATION OF THE RIPENING JAPANESE PERSIMMON<sup>1</sup>

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## INTRODUCTION

Although the Japanese persimmon (*Diospyros kaki*) was planted in California as early as 1870 (18),<sup>2</sup> no more than 200 acres seem to have been planted as late as 1919. During the 8-year period 1919-1926, however, the bearing acreage more than doubled and the nonbearing acreage increased about sixteenfold, indicating a renewed activity in the industry (11, 18).<sup>3</sup>

It is indeed surprising that this industry has not developed more rapidly. The Japanese persimmon is attractive in color, possesses a pleasant flavor, has a high food value, and contains vitamin C (19). In addition, disease seldom attacks either the tree or the fruit. The crop enters the market at a time when few other fresh fruits are available. That it keeps well in cold storage is indicated by the fact that near Peking, China, practically the whole winter supply is stored in large outdoor pits (6). Some of these desirable qualities have already been pointed out by Gore and Fairchild (8), and a more widespread knowledge of them should help to make this fruit more popular.

It is quite probable, however, that the difficulty of removing the astringent taste has been a serious drawback to the wider use of the fruit. In an attempt to eliminate this handicap, the United States Plant Introduction Gardens at Chico, Calif., has introduced a non-astringent variety, the Fuyu. Although its introduction may partly account for increased plantings, the Fuyu has not yet appeared in commercial quantities in the markets of southern California, where the bulk of the industry is located. Since, up to this time, the chief commercial varieties have been astringent, it appears that the removal of the astringency is still of importance.

In spite of the fact that means for removal of the astringency have been known for some time (8), and are employed in Japan, they seem as yet to be little used commercially in the United States. With the demonstration that ethylene would decrease the astringent taste and enhance the color (2), a new means of astringency removal seemed to be available. That this method is of some practical significance is indicated by the fact that of 550 tons of persimmons estimated as grown in southern California in 1927,<sup>4</sup> 25 tons were treated with ethylene for the local trade.

<sup>1</sup> Received for publication June 4, 1930; issued February, 1931.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 181.

<sup>3</sup> Calculated from data on trees, estimating 68 trees per acre

<sup>4</sup> Figures given in a personal communication from the Southern California Persimmon Grower's Association.

In addition to the above-mentioned changes in the color and taste of persimmons brought about by ethylene, preliminary studies (3) had suggested that it has a softening effect. Ethylene also seemed to increase the respiration rate,<sup>5</sup> as it does that of the lemon (5), and there appeared to be a relation between the effect of ethylene and the stage of maturity at which treatment is applied, similar to that reported by Rosa (17) for the tomato.

Related to the task of removing the astringent taste, therefore, is the determination of the proper stage of maturity at which the fruit should be picked and ethylene applied. Consequently the work reported here was undertaken to determine the effect of ethylene on coloring, softening, chemical composition, and respiration of Japanese persimmons of two varieties at different stages of ripening on the tree and during a short storage period.

### PROCEDURE

Samples of the Hachiya, the chief commercial, astringent variety in California, were obtained from an 8-year-old tree on a ranch at Fullerton, Calif. The fruit was picked in such a manner as to get as representative a sample as possible at different stages of maturity. As soon as the samples were picked they were carried by automobile directly to the laboratory. Samples of the nonastringent Fuyu were taken from one tree in the United States Plant Introduction Gardens at Chico, Calif., and sent by express to the laboratory, being 36 to 48 hours in transit. Sampling began September 15, when the fruit was almost wholly green but of nearly full size, and continued at weekly intervals until October 27.

On arrival at the laboratory the persimmons of each variety were divided as uniformly as possible in regard to size and color into four lots of 10 fruits each. The rate of respiration of lots A, B, and C was determined before treatment began, its determination requiring 15 to 22 hours. Lot D was kept at the same temperature as lot B until analyzed, and was therefore the check lot. Data were secured from this lot to determine the effect of temperature and other environmental conditions. After the respiratory rate had been obtained, the untreated lot, A, was analyzed to show maturity changes.

Lots B and C were then exposed to ethylene, 1 part in 1,000 parts, for 46 to 52 hours at room temperature, in large desiccators, which were aired and recharged daily. The respiration rate was then determined for lots B, C, and D. Lots B and D were immediately analyzed, and C was held for observation. Lot B, then, constituted the sample analyzed after treatment. (Table 1.)

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<sup>5</sup> DAVIS, W. B. Unpublished data.

TABLE 1.—Chemical composition of Japanese persimmons, before and after treatment with ethylene

## HACHIYA VARIETY

Sample No. <sup>a</sup>	Date of picking	Astringency <sup>b</sup>	Moisture	Percentage composition on a moisture-free basis					
				Insoluble solids	Soluble solids (by difference)	Total acid <sup>c</sup>	Sugars		
							Reducing <sup>d</sup>	Sucrose	Total
	1927		<i>Per cent</i>						
3A.....	Sept. 20	---	83.24	23.63	76.37	0.925	46.72	0	46.72
3B.....	do.	---	83.60	26.95	73.05	1.128	56.59	0	56.59
3D.....	do.	---	83.17	26.92	73.03	.980	55.58	0	55.56
5A.....	Sept. 27	---	82.57	20.37	79.63	.889	62.88	0	62.88
5B.....	do.	---	82.92	21.95	78.05	.878	63.76	0	63.76
5D.....	do.	---	82.34	21.86	78.14	.764	64.10	0	64.10
7A.....	Oct. 11	+++	80.63	16.93	83.07	.774	57.58	4.18	71.76
7B.....	do.	+	80.99	16.89	83.11	.763	57.12	3.79	70.91
7D.....	do.	+++	80.60	18.76	81.24	.748	66.50	3.51	70.01
10A.....	Oct. 18	+++	79.78	16.62	83.38	.692	71.17	2.57	73.74
10B.....	do.	+	80.62	14.71	85.29	.774	72.86	1.24	74.10
10D.....	do.	+++	79.94	16.20	83.80	.773	70.54	1.99	72.53
11A.....	Oct. 24	+++	79.40	14.47	85.53	.704	73.54	1.80	75.34
11B.....	do.	0	79.76	13.24	86.76	.692	73.07	1.78	74.85
11D.....	do.	++	78.56	13.85	85.15	.678	72.05	2.61	74.57
11E.....	do.	0	79.14	14.96	85.04	.527	71.19	3.07	74.26
11F.....	do.	+	79.50	13.07	86.93	.634	71.66	1.71	73.37
11N <sup>e</sup> .....	do.	++	79.02	15.59	84.41	.620	71.16	1.48	72.64
11M <sup>e</sup> .....	do.	+	79.16	14.15	85.84	.648	72.84	1.82	74.66
11L <sup>e</sup> .....	do.	+++	79.02	16.30	83.70	.543	71.21	2.53	73.74
11N <sup>f</sup> .....	do.	+++	78.25	14.02	85.98	.690	73.29	1.38	74.67
11M <sup>f</sup> .....	do.	0	78.70	14.18	85.82	.610	74.27	1.69	75.96
11L <sup>f</sup> .....	do.	+	78.36	14.28	85.72	.601	73.94	1.76	75.70

FUYU VARIETY <sup>g</sup>

2A.....	Sept. 15	(o)	84.40	22.71	77.29	0.674	66.07	0.90	86.97
2B.....	do.	(o)	84.74	19.14	80.88	.721	70.51	0	70.51
2D.....	do.	(o)	83.62	23.63	76.37	.678	71.31	0	71.31
4A.....	Sept. 22	---	84.00	19.81	80.19	.531	69.75	0	69.75
4B.....	do.	---	84.45	16.66	83.34	.579	71.77	0	71.77
4D.....	do.	---	83.78	19.48	80.52	.586	67.14	0	67.14
6A.....	Oct. 6	---	82.91	16.38	83.62	.497	71.62	3.98	75.60
6B.....	do.	---	83.13	15.77	84.23	.474	72.44	3.91	76.35
6D.....	do.	---	82.86	16.39	83.61	.496	71.76	3.85	75.61
9A.....	Oct. 13	---	81.78	16.30	83.70	.494	72.28	3.24	75.52
9B.....	do.	---	81.98	13.60	86.40	.472	73.36	4.61	77.97
9D.....	do.	---	82.09	15.25	83.75	.530	74.26	2.01	76.27
12A.....	Oct. 23	---	81.74	13.97	85.03	.438	75.36	2.52	77.88
12B.....	do.	---	82.03	13.24	86.76	.473	75.91	3.12	79.03
12D.....	do.	---	81.92	14.38	85.62	.498	75.78	2.05	77.83
13A.....	Oct. 27	---	79.16	12.09	87.91	.432	75.43	2.06	77.49
13B.....	do.	---	79.74	10.37	89.63	.395	75.17	5.08	80.25
13D.....	do.	---	79.16	11.52	88.48	.432	77.40	2.54	79.94
13E.....	do.	---	79.46	10.52	89.48	.438	76.73	4.82	81.55
13F.....	do.	---	79.60	12.11	87.89	.392	77.84	2.01	79.85
13N <sup>e</sup> .....	do.	---	78.86	9.74	90.26	.426	79.42	2.74	82.16
13M <sup>e</sup> .....	do.	---	79.51	10.59	89.41	.390	77.99	3.03	81.02
13L <sup>e</sup> .....	do.	---	78.74	11.48	88.52	.376	79.12	2.07	81.19

<sup>a</sup> A and N, untreated, analyzed immediately after respiratory activity was determined; B and M, treated with ethylene; D and L, check, held under same conditions as B, but not treated; E, treated with ethylene for 1 week; F, check, held under same conditions as E.

<sup>b</sup> + = slightly astringent; ++ = medium astringent; +++ = very astringent.

<sup>c</sup> As anhydrous citric.

<sup>d</sup> As invert.

<sup>e</sup> Stored at room temperature for 1 week.

<sup>f</sup> Stored at room temperature for 2 weeks.

<sup>g</sup> Nonstringent variety.



The respiratory rate was thus ascertained for two lots, B and C, before treatment and also after treatment. Since, however, there was little difference between the respiratory rates of lots B and C, either before or after treatment, the average of these two was plotted. (Fig. 4.) In Table 2, B designates the average of the two lots B and C after treatment, and G is the average of the same lots before treatment.

TABLE 2.—*Respiration of Japanese persimmons before and after treatment with ethylene*

HACHIYA VARIETY

Sample No. <sup>a</sup>	Date of picking	Carbon dioxide	Oxygen	Ratio carbon dioxide oxygen at 0° C. 760 m. m
		<i>C c per kilo per hour</i>	<i>C c per kilo per hour</i>	
	1927			
3A.....	Sept. 20.....	15.9	14.0	1.13
3D.....	do.....	25.3	22.8	1.11
3B.....	do.....	40.0	25.0	1.62
3G.....	do.....	15.0	14.6	1.04
Difference B—G.....	.....	25.0	10.4	.58
5A.....	Sept. 27.....	14.7	14.0	1.05
5D.....	do.....	10.5	10.7	.98
5B.....	do.....	34.2	21.1	1.62
5G.....	do.....	15.5	15.6	1.00
Difference B—G.....	.....	18.7	5.5	.62
7A.....	Oct. 11.....	19.9	20.3	.98
7D.....	do.....	24.8	24.4	1.22
7B.....	do.....	31.8	16.9	1.89
7G.....	do.....	17.4	17.0	1.02
Difference B—G.....	.....	14.4	—1	.87
10A.....	Oct. 18.....	17.2	15.5	1.11
10D.....	do.....	11.4	10.5	1.09
10B.....	do.....	28.7	20.1	1.43
10G.....	do.....	16.9	13.8	1.25
Difference B—G.....	.....	11.8	6.3	.18
11A.....	Oct. 24.....	17.4	15.9	1.09
11D.....	do.....	18.9	13.4	1.36
11B.....	do.....	32.0	18.9	1.69
11G.....	do.....	17.5	16.1	1.09
Difference B—G.....	.....	14.5	2.8	.60
11F.....	Oct. 24.....	23.4	6.5	3.60
11F.....	do.....	26.9	19.0	1.49
Difference E—F.....	.....	—3.5	—12.5	2.11
11L <sup>b</sup> .....	Oct. 24.....	15.0	10.4	1.43
11M <sup>b</sup> .....	do.....	26.4	18.6	1.42
11N <sup>b</sup> .....	do.....	16.5	15.1	1.09
Difference M—N.....	.....	9.9	3.5	.33
11L <sup>c</sup> .....	Oct. 24.....	21.2	14.7	1.44
11M <sup>c</sup> .....	do.....	25.2	12.5	2.01
11N <sup>c</sup> .....	do.....	19.1	16.7	1.14
Difference M—N.....	.....	6.1	—4.2	.87

FUYU VARIETY

2A.....	Sept. 15.....	14.4	13.9	1.04
2D.....	do.....	11.1	13.1	.85
2B.....	do.....	29.9	24.9	1.20
2G.....	do.....	14.6	14.5	1.01
Difference B—G.....	.....	15.3	10.4	.19
4A.....	Sept. 22.....	14.3	13.7	1.04
4D.....	do.....	10.9	10.5	1.04
4B.....	do.....	26.4	21.5	1.23
4G.....	do.....	14.1	13.1	1.08
Difference B—G.....	.....	12.3	8.4	.15
6A.....	Oct. 6.....	13.6	14.1	.97
6D.....	do.....	11.7	10.1	1.16
6B.....	do.....	24.8	20.1	1.23
6G.....	do.....	13.5	13.6	1.00
Difference B—G.....	.....	11.3	6.5	.23

<sup>a</sup> A, untreated; B and M, average of 2 lots, B and C, after treatment with ethylene; D and L, checks, held under same conditions as B but not treated; E, treated with ethylene for 1 week; F, check, held under same conditions as E but not treated; G and N, average of 2 lots, B and C, before treatment with ethylene.

<sup>b</sup> Stored at room temperature for 1 week.

<sup>c</sup> Stored at room temperature for 2 weeks.

TABLE 2.—*Respiration of Japanese persimmons before and after treatment with ethylene—Continued*

FUYU VARIETY—Continued

Sample No.	Date of picking	Carbon dioxide	Oxygen	Ratio carbon dioxide oxygen at 0° C. 760 m. m.
		<i>C c. per kilo per hour</i>	<i>C c. per kilo per hour</i>	
	1927			
9A.....	Oct. 13.....	13.3	13.4	0.99
9D.....	do.....	9.4	10.2	.92
9B.....	do.....	23.2	20.9	1.11
9G.....	do.....	13.4	13.3	1.00
Difference B—G.....	do.....	9.8	7.6	.11
12A.....	Oct. 23.....	13.0	12.9	1.01
12D.....	do.....	10.2	9.2	1.11
12B.....	do.....	25.9	19.5	1.33
12G.....	do.....	10.8	12.0	.90
Difference B—G.....	do.....	15.1	7.5	.43
13A.....	Oct. 27.....	14.9	15.1	.99
13D.....	do.....	12.2	10.4	1.17
13B.....	do.....	22.1	16.9	1.31
13G.....	do.....	13.6	12.8	1.06
Difference B—G.....	do.....	8.5	4.1	25.23
13E.....	Oct. 27.....	18.9	8.3	2.28
13F.....	do.....	11.9	10.1	1.18
Difference E—F.....	do.....	7.0	1.8	1.10
13L <sup>b</sup> .....	Oct. 27.....	12.6	12.0	1.05
13M <sup>b</sup> .....	do.....	21.4	17.9	1.20
13N <sup>b</sup> .....	do.....	11.7	10.8	1.08
Difference M—N.....	do.....	9.7	7.1	.12

<sup>b</sup> Stored at room temperature for 1 week.

When the fruit was considered commercially mature, a large number of fruits were picked for storage tests as well as for maturity tests. The persimmons used for the storage tests were divided at the beginning of the storage period into lots of eight or nine fruits each designated as L, M, and N corresponding in treatment to lots D, B, and G mentioned above. These were stored at room temperature, some for one week and some for two weeks. (Tables 1 and 2.) Ethylene was applied for 46 to 52 hours at the end of the storage period. Only one lot was used to determine respiration after treatment.

Lots 11E and 13E were treated with ethylene for one week to determine the effect of ethylene over a longer period than that required for maturity tests. Lots 11F and 13F were held under the same conditions as untreated checks.

Temperatures during the period of treatment were recorded by means of thermographs. The maximum temperatures for all lots ranged from 20.2° to 27° C.; the average maximum was 21.7°. The range of minimum temperatures was from 14° to 19°, the average minimum being 16.6°.

## ANALYTICAL METHODS

In determining color, the percentage of yellow surface as compared to green surface was estimated by eye for each fruit. Each analyst made independent observations, and from these the average for the whole sample was calculated.

The pressure was measured by means of the instrument devised by Magness and Taylor (15) for pears and apples, the plunger head being

seven-sixteenths of an inch in diameter. Two determinations on each of several unpeeled fruits were made, and the average was taken.

In preparing the samples for analysis, 10 fruits were cut from stem to stylar end, and half of each was discarded. The other halves, unpeeled, were then seeded, if necessary, and ground in a food chopper, first through a medium cutter and then through a nut-butter cutter. When this grinding proved unsatisfactory for the firm fruits they were grated on the fine teeth of a tin household grater.

The determinations of moisture and total solids were made on the same sample, by drying it to constant weight in vacuo at 70° C., according to official methods of the Association of Official Agricultural Chemists (1, p. 210, No. 5).

Insoluble solids were determined as follows: Twenty-five grams of the sample was boiled in 200 c. c. of distilled water for 30 minutes. After standing overnight, the material was filtered through closely woven muslin and thoroughly washed with water, and the residue was dried to constant weight at 100° C.

The quantity of soluble solids was determined by calculating the difference between total and insoluble solids.

Hydrogen-ion concentration, expressed as pH, was determined according to the LaMotte indicator method by matching the diluted juice (3 volumes of water to 1 of juice) against color standards of known value. Juice was obtained by squeezing the macerated pulp through several folds of cheesecloth.

For the determination of titratable acidity, 25 gm. of the pulp was boiled in 200 c. c. of distilled water for 30 minutes, filtered, washed, cooled, and titrated against 0.1562 normal sodium hydroxide (1 c. c. was equivalent to 0.01 gm. anhydrous citric acid), phenolphthalein being used as an indicator. Owing to the color in the Hachiya variety, it was necessary with this fruit to use a spot plate in determining the endpoint.

The following procedure was used in preparing the sample for the estimation of sugars: Ten grams of the pulp, into which approximately 0.5 gm. of calcium carbonate had been mixed, was boiled in 150 to 200 c. c. of 95 per cent alcohol for 30 minutes. This extraction was repeated twice with 80 per cent alcohol and once with distilled water. After evaporation of the alcohol, reducing sugars were determined according to official methods of the Association of Official Agricultural Chemists (1, p. 190, No. 35); the Munson and Walker method was used for reduction, and the cuprous oxide was titrated according to the volumetric permanganate method (1, p. 192, No. 39). Inversions were made by treatment with concentrated hydrochloric acid at room temperature for 24 hours or more. The quantity of sucrose was calculated by multiplying the difference between reducing sugars before and after inversions by the factor 0.95.

The respiratory activity was determined according to the method described by Johnstone (9) by which the samples were kept in an atmosphere which was approximately free from carbon dioxide and to which oxygen was supplied as it was removed. The quantity of oxygen absorbed by the water in the oxygen reserve cylinder was estimated to be 3 per cent of the water volume. Before they were immersed the respiratory chambers containing the fruit were brought approximately to the temperature of the water bath, kept at 25° C. by an electrically operated mercury thermoregulator.

From 100 to 200 c. c. of approximately normal sodium hydroxide served to absorb the carbon dioxide in each chamber. In most cases this quantity was sufficient to prevent any difficulty in titrating by the double-titration method, but occasionally it was necessary to use comparison standards for both end points.

### RESULTS

The Hachiya appears to be physiologically the more active variety. The results from lots designated A (untreated) in Table 1 taken to follow maturity changes occurring with advance of the season clearly indicate this. Color (figs. 1 and 2), total sugars, and weight (fig. 3)

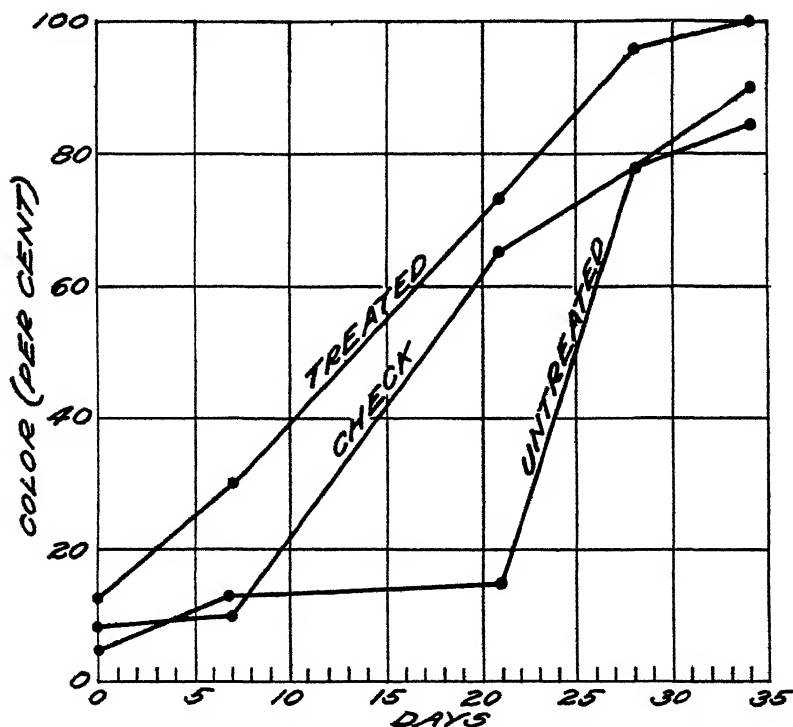


FIGURE 1.—Increase in color of Hachiya persimmons before and after treatment with ethylene during the sampling period.

showed a greater increase and sucrose a greater decrease in the Hachiya. Moisture and insoluble solids seemed to decrease more in the Fuyu, but there was little difference in acidity changes. There was little change in either hardness or astringent taste, even though the last samples were picked after the fruits were fairly well colored.

The two varieties exhibited other differences. They differed in shape, and the Fuyu was smaller and colored more slowly than the Hachiya. The Fuyu possessed a larger quantity of total sugar, reducing sugar, soluble solids and moisture, but the Hachiya had the greater quantity of acid.

An interesting result was the sudden appearance of sucrose. With the exception of the untreated Fuyu in the first lot, no sucrose

appeared in either variety until the third week of sampling, October 6-11. After this the quantity in both varieties gradually decreased. Other workers seem to find sucrose present in the Japanese persimmon (8, 16, 18). Komatsu and Ishimasa (12) report an increase of sucrose in one variety of Japanese persimmon as it ripened.

The greater respiratory activity of the Hachiya revealed by the data which have been reduced to standard conditions of 760 mm. and 0° C. is shown in Figure 4 in the upper set of curves, which were plotted from triplicate lots. The quantities of oxygen intake and carbon dioxide output were consistently larger in the Hachiya. Possibly a little more oxygen, relative to carbon dioxide output, was taken in by the Fuyu, as a comparison of the respiratory ratios suggests. The peaks of the curves for the Hachiya were reached October

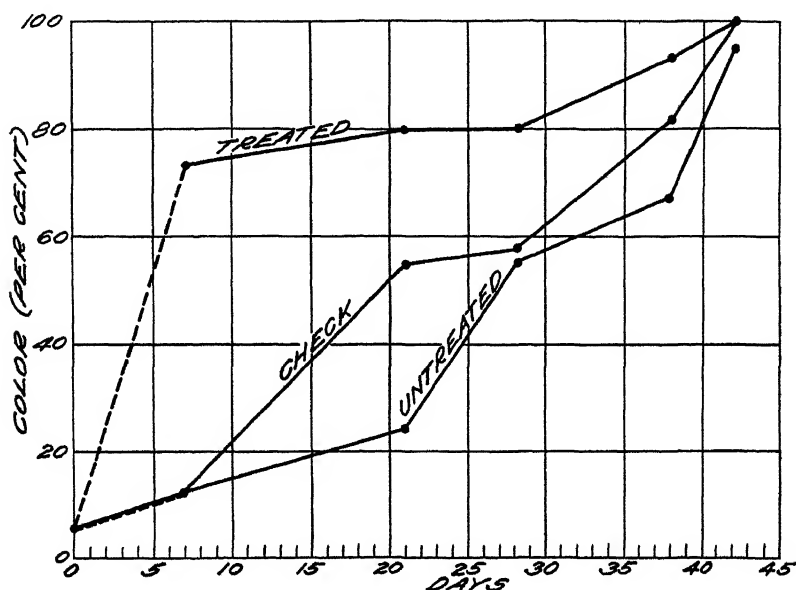


FIGURE 2.—Increase in color of Fuyu persimmons before and after treatment with ethylene during the sampling period

6-11, but the curves for the Fuyu show little rise or fall. In considering the effect of ethylene, this physiological difference should be noted.

In order to determine the effect of ethylene, the check and treated lots were compared. Student's method (13, 14) of calculating odds appeared to find particular application here, and accordingly was used as an aid in determining the significance of the results. When the curves for the treated and check lots were practically the same over part of the curve but diverged for the remainder, the odds were calculated in two ways—for the whole period, and for the period of divergence only. In no case did the odds reach 30 to 1, below which differences are not regarded as significant by either method of calculation.

The most pronounced effect of ethylene treatment was to cause a rapid softening (fig. 5), a change readily produced at any of the stages of maturity studied. The data for all treated lots were alike in that they lay entirely outside the range of the checks. It is probable that the after-treatment data record only the toughness of the peel.

Next in importance to the softening effect of ethylene was its effect on the color, which was considerably increased in the Hachiya. In

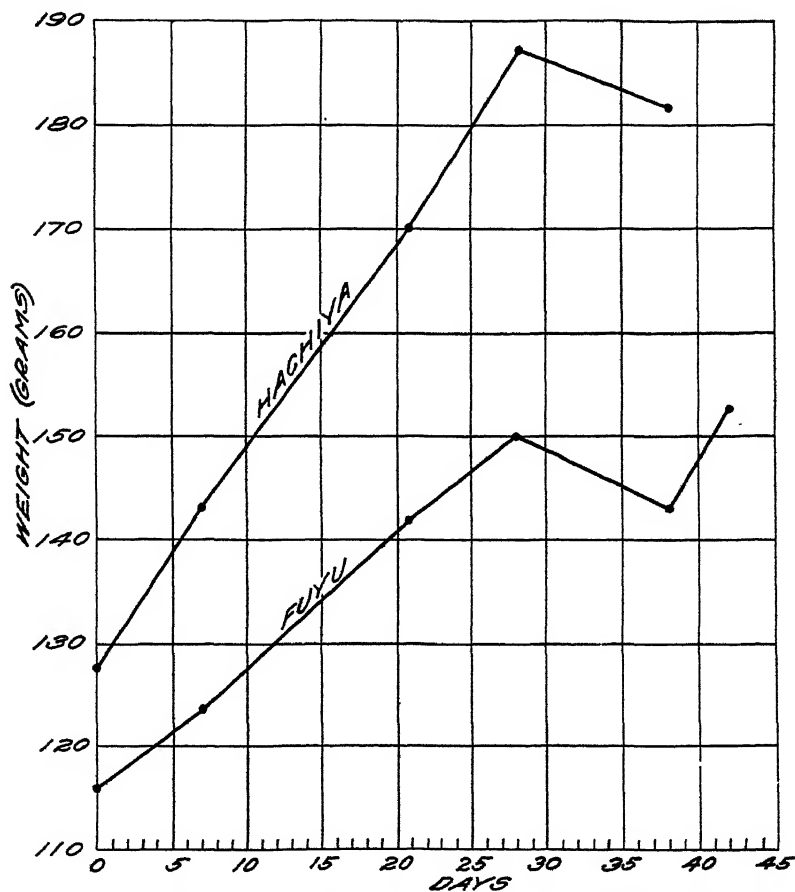


FIGURE 3.—Increase in weight of Hachiya and Fuyu persimmons during the sampling period

the Fuyu the odds for increased color are rather low, 34 to 1, but it is possible that a longer treatment is necessary for this less active fruit, and the method of estimating color should also be taken into consideration.

Significant changes occurred in three other constituents as a result of the treatment. In the Fuyu the insoluble solids decreased and simultaneously the soluble solids increased (figs. 6 and 7), but it is uncertain whether or not the same changes took place in the Hachiya. The third constituent affected was moisture (fig. 6) in the Hachiya,

and though the differences were rather small, 0.2 to 1 per cent, they were consistent, yielding high odds. There is not enough difference between the curves for the Fuyu to indicate with certainty the same increase in moisture.

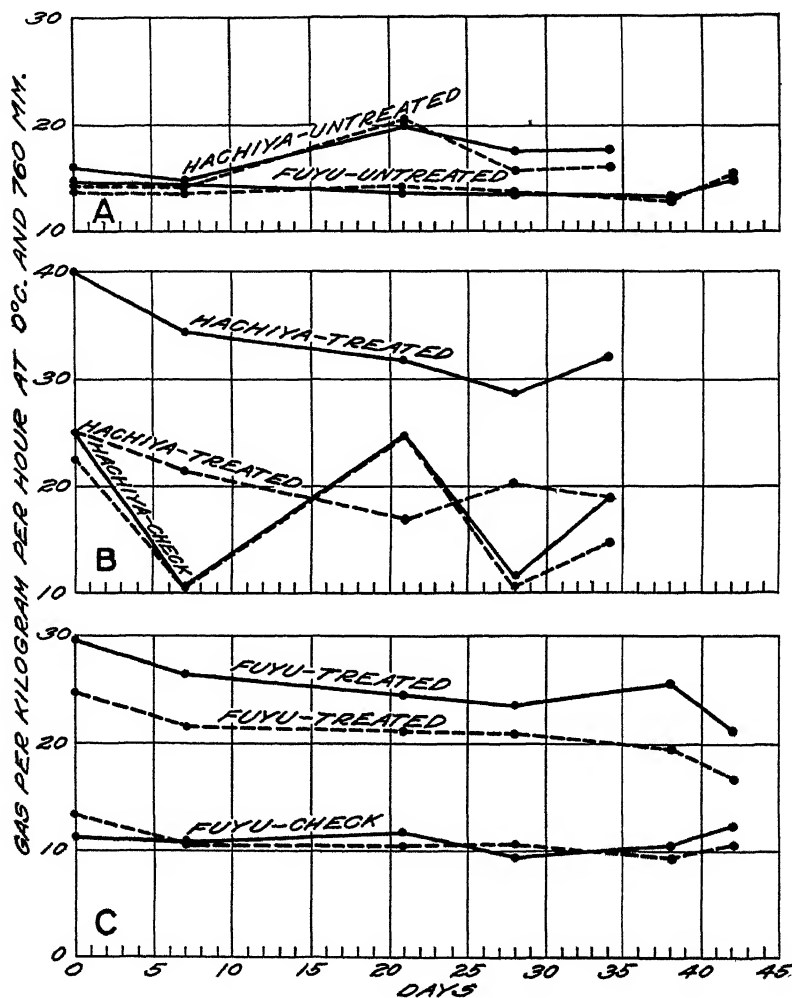


FIGURE 4.—Changes in respiratory activity of Hachiya and Fuyu persimmons: A, with advance of season; B and C, after treatment with ethylene. Solid lines represent carbon dioxide given off by fruit; broken lines the oxygen taken in

The odds for increase of sucrose, reducing sugar, or total sugars after treatment are too low to be significant for either variety. (Table 3 and fig. 8.) In fact, the odds are not such as to indicate definitely the effect of ethylene on the sugars. These results, however, do suggest the need for further study of the effect of ethylene on this constituent.

TABLE 3.—Effect of ethylene treatment upon sugar content of Japanese persimmons (dry-weight basis)

Date of picking	Hachiya variety			Fuyu variety		
	Check lot	Treated lot	Difference	Check lot	Treated lot	Difference
REDUCING SUGARS						
1927	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Sept. 15, 20.....	55.56	56.59	+1.03	71.31	70.51	-0.80
Sept. 22, 27.....	64.10	63.76	-.34	67.14	71.77	+4.63
Oct. 6, 11.....	66.60	67.12	+.62	71.76	72.44	+.68
Oct. 13, 18.....	70.54	72.86	+2.32	74.26	73.36	-.90
Oct. 23, 24.....	72.06	73.07	+1.01	75.78	75.91	+.13
Oct. 27.....				77.40	75.17	-2.23
SUCROSE						
Oct. 6, 11.....	3.51	3.79	+.28	3.85	3.91	+.06
Oct. 13, 18.....	1.99	1.24	-.75	2.01	4.61	+2.60
Oct. 23, 24.....	2.61	1.78	-.83	2.05	3.12	+1.07
Oct. 27.....				2.34	5.08	+2.54
TOTAL SUGARS						
Sept. 15, 20.....	55.56	56.59	+1.03	71.31	70.51	-.80
Sept. 22, 27.....	64.10	63.76	-.34	67.14	71.77	+4.63
Oct. 6, 11.....	70.00	70.91	+.91	75.61	76.35	+.74
Oct. 13, 18.....	72.53	74.10	+1.57	76.27	77.97	+1.70
Oct. 23, 24.....	74.67	74.85	+.18	77.82	79.02	+1.20
Oct. 27.....				79.94	80.26	+.32

Ethylene affected the respiratory activity of both varieties of persimmons in a marked degree. (Table 2.) The oxygen intake and carbon dioxide output, especially the latter, were greatly stimulated, as may be seen in the two lower sets of curves in Figure 4. Two exceptions occurred, in lots 3D and 7D, but the increase in these checks may have been due to accidental exposure of the fruit to ethylene in airing the desiccators. In the more active Hachiya, the maximum production of carbon dioxide (fig. 4) showed an increase of 167 per cent. Oxygen intake did not keep pace with carbon dioxide evolved, which resulted in a tendency for the respiratory ratio,  $\frac{\text{CO}_2}{\text{O}_2}$ , to increase in the later stages of ripening, especially in the Hachiya. That longer periods of treatment would have further increased this ratio is indicated by the results of treating lots 11E and 13E for 168 hours. (Table 2.)

The stimulative effect of ethylene depends upon the stage of maturity of the fruit to which it is applied, decreasing as the fruit becomes more mature. This decrease appears to bear no direct relation to the respiratory changes taking place during maturation.

An attempt made to follow the changes in astringency accurately by the official tannin method (1, p. 340, No. 18) for tea, was so unsatisfactory that only the results secured roughly by tasting are given. These show that the astringent taste decreased after treatment. (Table 1.) The total acidity was not greatly affected by ethylene, as may be seen in Figure 8. The pH values of water extracts of the fruits were determined, but they varied so little that the data are not given.



Unfortunately little attention could be given to storage experiments, but the results indicate that there was little deviation from the trend of changes taking place previous to storage. The decrease in astringent taste, however, was evident in the stored fruit.

### DISCUSSION

According to the data for the two varieties studied, the chief effect of ethylene is on hardness, color development, and respiration, particularly the first. This softening effect is apparently not related to the stage of maturity at which ethylene is applied, quite in contrast to the effect on color and respiration. The fact that the

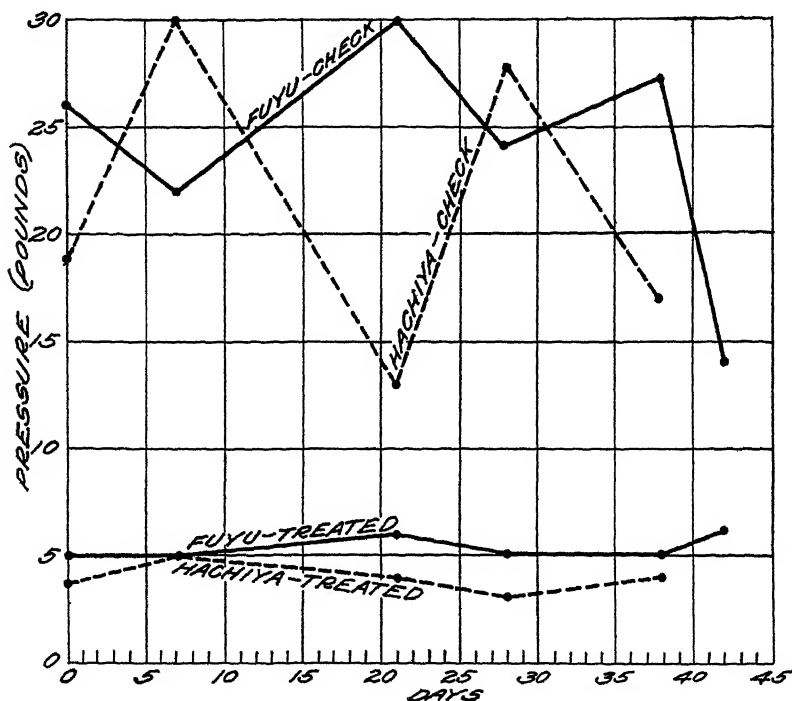


FIGURE 5.—Changes in hardness of Fuyu and Hachiya persimmons before and after treatment with ethylene, during the sampling period. Pressure is given in pounds necessary to penetrate skin with a  $\frac{7}{16}$ -inch head

persimmon does not normally become softer with the advance of the season probably renders the pressure test of little value in determining maturity.

Associated with the softening there is an increase of soluble solids and decrease of insoluble solids as a result of treatment with ethylene. The change is unquestionable for the Fuyu, and though the odds are so low as to be doubtful for the Hachiya, nevertheless both the direction and shape of the curves suggest that the same change may have taken place. The increase of soluble solids would not be so great in the more active Hachiya if the soluble material was removed by respiration.

These data do not indicate that increase of soluble solids is due to increase of total acids and total sugars. Rather it seems more likely to be caused by changes in pectic substances or hemicelluloses, but unfortunately lack of time prevented the determination of these substances.

Harvey (10) reports an increase in alcohol-ether-soluble material in the epicotyl of the sweet pea after ethylene treatment. High odds for both increased soluble solids and osmotic pressure were revealed

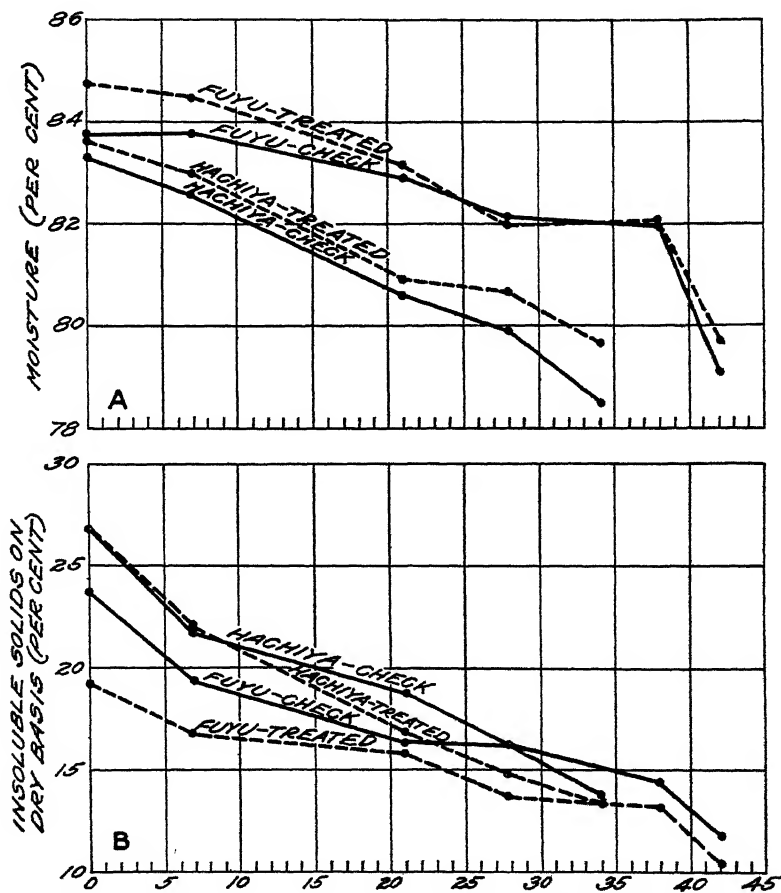


FIGURE 6.—Changes in moisture (A) and in insoluble solids (B) of Hachiya and Fuyu persimmons before and after treatment with ethylene during sampling period

when his data had been calculated for odds, but ethylene decreased the respiration rate. It seems rather difficult, however, to compare two tissues so diverse in nature and especially so diverse in their response to environmental conditions.

Ethylene brought about an increase in color even in the very immature fruit, although such fruit later became discolored. Experience has shown that longer treatment of immature fruit does not produce a well-colored product, nor will fruit picked too green and

held in storage for some time color afterward if treated. Although the estimation of color is rather difficult and color as a standard of maturity is hard to apply, it is clear that better results are obtained in artificial coloring of persimmons if they are picked at the proper stage of maturity.

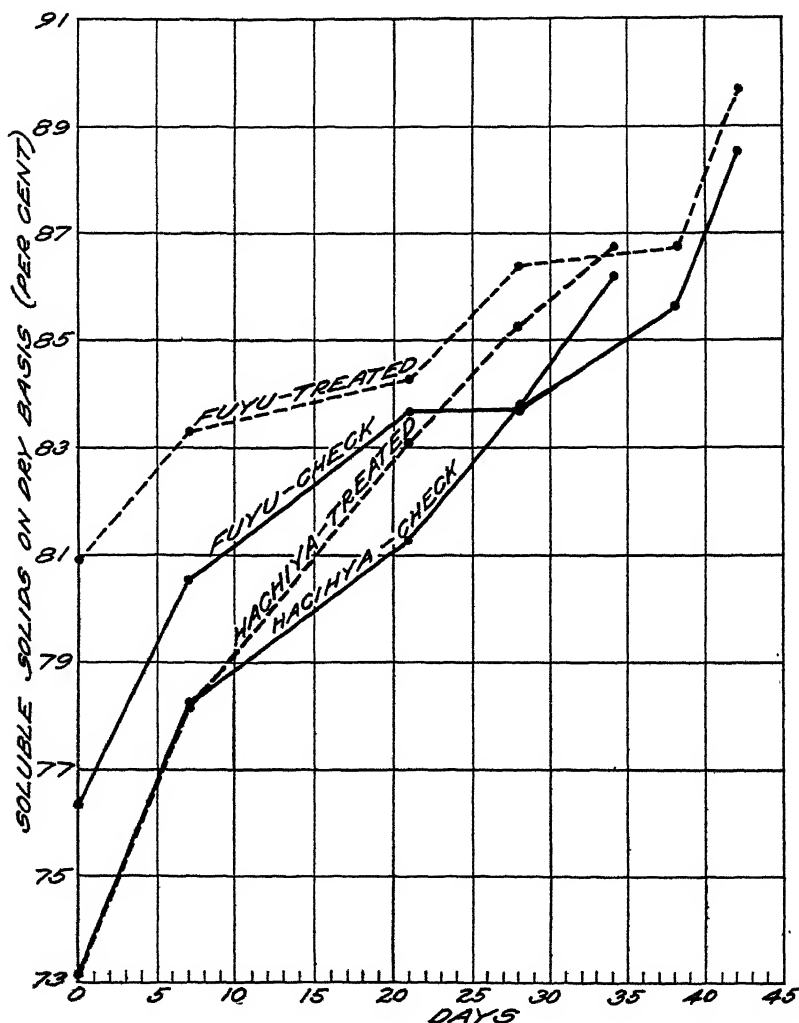


FIGURE 7.—Changes in soluble solids (dry basis) of Hachiya and Fuyu persimmons before and after treatment with ethylene during the sampling period

The data here reported indicate that fruit 80 per cent colored has more than 95 per cent of the total sugars and nearly the maximum weight of the ripe fruit. These data, however, are the results of but one season's study, and might not be obtained another season, as seasonal fluctuations in behavior are known to occur in other fruits, as for example the pomegranate (4). Total sugars in these persim-

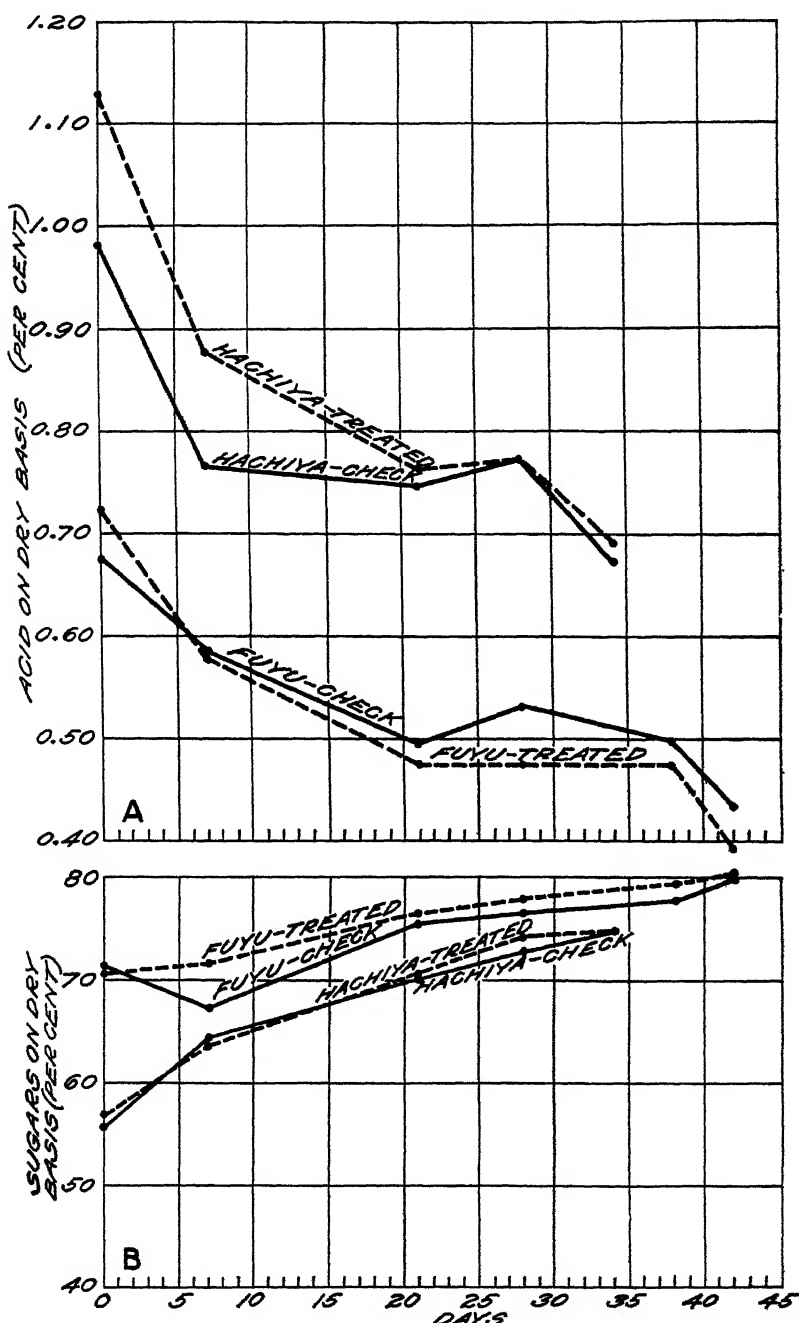


FIGURE 8.—Changes in acid (A) and in total sugars (B) (dry basis) of Hachiya and Fuyu persimmons before and after treatment with ethylene during the sampling

mons reached a maximum rather early, but this might not occur another season.

The importance of the greater physiological activity of the Hachiya revealed by these studies should be noted. If increased respiration explains the increased moisture of the treated Hachiya, then the lesser physiological activity of the Fuyu may explain why the odds for increased moisture and color development are so low for this variety. A longer exposure may be necessary to obtain the same degree of color and moisture increase as for the Hachiya. Age and climate may also complicate these results.

Although the softening effect, the most striking result of ethylene treatment of persimmons, may limit the use of ethylene to treatment of fruits intended for local distribution, the enhancement of color and decrease in astringent taste, making a more uniform, marketable, and palatable product, seem to be of practical advantage.

The results of these studies do not indicate any one easily explained cause of the effect of ethylene on persimmons. Since the respiratory rate is disturbed, the effect seems to be on the general metabolism with subsequent changes in chemical constituents. Further investigation of these softening and color changes is of great importance to the fruit and vegetable industry.

#### SUMMARY

Attention is directed to the desirable qualities of Japanese persimmons, their increased production, and the practical advantages of treating them with ethylene.

A study of some physical, chemical, and respiratory changes occurring during the growth and ripening of a nonastringent and an astringent variety of Japanese persimmon, and of the effect of a low concentration of ethylene, applied at intervals during maturation, on the changes mentioned, is reported.

The period of study extended from September 15, when the fruit was almost entirely green but of nearly full size, to October 27, 1927, when it was considered commercially mature.

It was found that physiologically the Hachiya is more active than the Fuyu. Color, total sugars, reducing sugars, and weight showed a greater increase and sucrose a greater decrease in the Hachiya. Moisture and insoluble solids decrease, however, was greater in the Fuyu. The respiratory activity of the two varieties also differed, both in amount of gaseous exchange and in fluctuations in activity with the season's advance.

The Fuyu has a greater quantity of sugar, soluble solids, and moisture, but the Hachiya appears slightly more acid. The Fuyu develops color more slowly than the Hachiya.

Ethylene treatment stimulated softening, color development, and respiratory activity in both varieties. Astringent taste decreased and moisture increased in the Hachiya, and in the Fuyu insoluble solids decreased simultaneously with increase of soluble solids.

The stimulative effect of ethylene on respiration declined, and the respiratory quotient  $\frac{\text{CO}_2}{\text{O}_2}$  showed a tendency to increase as the fruit ripened, especially in the last stages and in the stored fruit.

Results of storing the less active mature persimmons, untreated, at room temperatures indicate for most constituents, except astringent taste in the Hachiya, which decreased rapidly, a continuance of the changes already begun on the tree.

Ethylene seems to act on the varieties of persimmons that were studied through its effect on the general metabolism.

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# THE MICROFLORA OF A RICH SULPHATE-CONTAINING SOIL<sup>1</sup>

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## CHARACTER OF THE SOIL

Some alkali soils are extremely rich in sulphates but contain only small quantities of other salts. Such a soil was used in this study. Areas within the district from which the soil was obtained were so high in soluble salts, mainly sodium sulphate, that only a few alkali-tolerant plants grew upon it. However, the surrounding soil, which contains only a low salt content, is highly productive. The samples were taken from the "alkali spots." Ten-kilogram portions were leached for two years.<sup>2</sup> Ninety liters of water passed through the soil, which, in addition to small quantities of other salts, removed 67.8 gm. of sodium sulphate and 12.7 gm. of sodium chloride.

After the soil was leached one crop of crimson clover and two crops of barley were grown on it. However, the yields in all cases were only slightly greater on the leached than on the unleached soil. The soil was sampled after each crop and its ammonifying, nitrifying, and nitrogen-fixing powers determined. The leaching of the soil greatly increased the number and activity of the microorganisms within it.<sup>3</sup> Its nitrogen-fixing powers were very active, for when kept in pots in the greenhouse with optimum moisture and nearly optimum temperature for a period of three years it gained nitrogen at the rate 1,142 pounds per acre-foot of soil.<sup>4</sup>

## METHOD OF STUDY

The soil for this study was taken from the pots after the second crop of barley had been harvested. It was carefully ground and mixed under conditions such that foreign organisms did not enter. It was then plated on synthetic Ashby and nutrient agar in dilutions of 1 to 20,000 and 1 to 200,000. The average results were: On nutrient agar, 5,600,000 colonies; on synthetic agar, 3,878,000 colonies; and on Ashby agar, 3,855,000 colonies. Apparently the microflora of this soil more nearly approach those of the synthetic alkali soil<sup>5</sup> than they do those of the chloride-containing soil, for in the sulphate and synthetic alkali leached soil the counts on the Ashby agar were low, whereas the chloride-leached soil yielded high counts on the Ashby agar. Both soils were of the same geological origin and were quite similar in physical and chemical properties, except that the soil considered in this paper contained mainly sulphates before leaching,

<sup>1</sup> Received for publication Sept. 19, 1930; issued February, 1931. Contribution from the Department of Chemistry and Bacteriology, Utah Agricultural Experiment Station.

<sup>2</sup> GREAVES, J. E. THE MICROFLORA AND PRODUCTIVITY OF LEACHED AND NONLEACHED ALKALI SOILS. *Soil Sci.* 23: 271-302. 1927.

<sup>3</sup> GREAVES, J. E. *Op. cit.* (See footnote 2.)

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<sup>5</sup> GREAVES, J. D. THE MICROFLORA OF LEACHED ALKALI SOILS: A LEACHED SODIUM-CHLORIDE SOIL. *Soil Sci.* 29: 79-83. 1930.



whereas the synthetic alkali soil contained before leaching equal quantities of sodium sulphate, sodium chloride, and sodium carbonate.

The different colony types appearing on the Ashby agar were obtained in pure cultures and studied morphologically and physiologically, as described in preceding papers.<sup>6</sup> Twenty-one organisms were studied in detail (Table 1), but only the more active ammonifiers and nitrogen fixers (16 in number) are discussed.

TABLE 1.—Summary of chief physiological and morphological properties of micro-organisms<sup>a</sup> obtained from an alkali soil rich in sodium sulphate

Culture No.	Nitrate reduction	Gelatin liquefaction	Starch hydrolysis	Milk peptonization	Indol production	Litmus-milk reaction	Action on glucose	Action on lactose	Action on sucrose	Shape <sup>b</sup>	Motility	Gram reaction	Chromogenesis	Spore formation	Ammonification	Nitrogen fixation
201B.....	—	+	+	+	+	—	+	0	+	F	—	+	—	+	2.6	2.8
202A.....	—	+	+	+	+	—	+	0	0	C	—	—	—	—	2.0	0
202B.....	+	+	+	+	+	—	+	0	0	F	—	—	—	—	2.8	4.2
202C.....	—	+	+	+	+	0	+	0	0	F	—	+	—	—	0	1.4
203A.....	—	+	+	+	+	—	+	0	0	F	—	+	—	—	6.1	1.4
208A.....	+	+	+	+	+	—	+	0	0	F	—	+	—	—	9.9	2.8
210B.....	—	+	+	+	+	0	+	0	0	F	—	+	—	—	9.9	0
210D.....	—	+	+	—	—	—	+	0	0	F	—	+	—	—	7.0	0
212C.....	—	+	+	—	+	0	0	+	0	F	—	+	—	—	0	2.8
212E.....	—	+	+	+	—	—	0	0	0	F	—	+	—	—	0	2.8
214.....	—	+	—	—	+	+	+	+	+	F	—	+	—	—	1.9	1.4
217.....	+	+	+	—	—	—	0	0	0	F	—	+	—	—	0	2.8
219A.....	—	+	+	—	—	+	+	0	0	C	—	+	—	—	1.2	2.8
219B.....	—	+	+	+	+	—	0	0	0	F	—	+	—	—	14.6	0
221B.....	+	+	+	+	+	—	0	0	0	F	—	+	—	—	9.9	2.8
223.....	+	+	+	+	+	—	+	0	+	F	—	+	—	—	25.9	0
225A.....	—	+	+	+	+	—	+	0	0	F	—	+	—	—	2.8	4.5
225C.....	+	+	+	—	+	+	+	+	0	F	—	+	—	—	7.1	1.4
226.....	—	+	+	+	+	—	+	0	0	F	—	+	—	—	8.5	0
227A.....	+	+	+	—	—	—	0	0	0	F	—	+	—	—	1.5	7.0
228B.....	+	+	+	—	—	—	0	0	0	F	—	+	—	—	4.8	0

<sup>a</sup> All given here are aerobic.

<sup>b</sup> F=filamentous, C=cocci, B=bacilli.

## CULTURAL CHARACTERS OF THE ORGANISMS

### FUNGI

**CULTURE 201B.**—Mycelium septate with slight branching. Conidiophore with conical masses as well as chlamydospores. Abundant growth on ordinary laboratory media. Liquefies gelatin. Rapidly peptonizes milk, with alkaline reaction. Does not reduce nitrates, but produces gas on potassium nitrate broth. No soluble pigment. Produces indol; hydrolyzes starch. Produces acid on glucose and sucrose without the production of gas. Aerobic. Produces 2.6 mgm. of ammonia in peptone and fixes 2.8 mgm. of nitrogen in soil containing mannite.

**CULTURE 203A.**—Mold. Mycelium septate, with branching sporangia and chlamydospores. Rapid growth on gelatin with liquefaction and with browning of media. Abundant growth on agar and in broth. Abundant white growth with bluish-green borders on potato. Does not produce indol nor reduce nitrates. Produces acid on glucose without the production of gas. Hydrolyzes starch. Aerobic. Produces 6.1 mgm. of ammonia in peptone and fixes 1.4 mgm. of nitrogen in soil.

**CULTURE 219B.**—Mold. Mycelium septate and branched. Serial mycelium and conidia. Abundant bluish-green growth on agar. Rapid liquefying growth on gelatin with browning of the media. Abundant bluish-green filamentous growth on potato. Peptonization of milk, with alkaline reaction. Produces indol and hydrolyzes starch. Does not produce acid on carbohydrate media. Aerobic. Produces 14.6 mgm. of ammonia in peptone but does not fix nitrogen.

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**CULTURE 225A.**—Mold. Mycelium septate and branched. Sporangia formed as in *Mucor*. Aerial mycelium nonseptate. Abundant blue-green growth on gelatin and potato. Gelatin liquefied. Moderate growth on agar and in broth. Peptonizes milk, with alkaline reaction. Produces indol but does not reduce nitrates. Produces acid on glucose and sucrose but not on lactose. Hydrolyzes starch. Aerobic. Produces 8.8 mgm. of ammonia in peptone and fixes 4.5 mgm. of nitrogen in soil.

**CULTURE 227A.**—Actinomyces. Mycelium straight with very little branching. Rapid and abundant growth on agar, broth, and gelatin. Liquefies gelatin. Moderate white growth with darkening on potato. Peptonizes milk with the production of acid. Produces indol, reduces nitrates, and hydrolyzes starch. Produces acid on glucose and actively decomposes cellulose. Aerobic. Produces small quantities of ammonia in peptone and fixes 7.0 mgm. of nitrogen in soil.

#### BACTERIA

**CULTURE 202B.**—Gram-negative, nonmotile, nonspore-forming; rods 0.5 to 0.7 by 1.2 to 1.9  $\mu$ , occurring singly and in clusters. Grows rapidly on agar. Grows slowly on gelatin, with liquefaction. Makes abundant tan-colored growth on potato. Slowly peptonizes litmus milk, with alkaline reaction. Reduces litmus. Produces indol. Reduces nitrates, hydrolyzes starch, and produces acid in glucose media. Aerobic. Produces 2.3 mgm. of ammonia in peptone and fixes 4.2 mgm. of nitrogen in soil.

**CULTURE 208A.**—Gram-positive; rods, 0.7 to 0.8 by 4.0 to 10.0  $\mu$ , occurring singly and in pairs. Motile by means of peritrichous flagella. Produces central ellipsoidal spores 0.8 by 1.5  $\mu$ . Grows rapidly on gelatin, with liquefaction. Makes abundant greenish-brown growth on agar and good growth in broth and on potato. Peptonizes milk, with alkaline reaction. Produces indol, reduces nitrates, produces acid on glucose and sucrose. Hydrolyzes starch and liberates hydrogen sulphide from peptone. Facultative aerobe. Produces 7 mgm. of ammonia from urea and 9.7 mgm. from peptone. Fixes 2.8 mgm. of nitrogen in soil.

**CULTURE 210B.**—Gram-positive, nonspore-forming; rods 0.7 to 0.8 by 3.3 to 4.5  $\mu$ , occurring singly, in pairs, and in short chains. Motile by means of peritrichous flagella. Makes abundant growth on all ordinary laboratory media. Rapidly liquefies gelatin. Causes no change in milk. Produces indol but does not reduce nitrates; produces acid on glucose, and hydrolyzes starch. Aerobic. Produces 9.9 mgm. of ammonia in peptone but does not fix nitrogen.

**CULTURE 210D.**—Gram-positive, nonspore-forming; rods 0.8 to 1.2 by 2.9 to 7.5  $\mu$ , occurring singly, in pairs, and in chains. Motile by means of peritrichous flagella. Makes abundant growth on agar, gelatin, and broth, but no growth on potato. Rapidly liquefies gelatin and turns milk alkaline. Does not form indol, nor reduce nitrates. Produces acid on glucose and hydrolyzes starch. Aerobic. Produces 7 mgm. of ammonia in peptone but does not fix nitrogen.

**CULTURE 212C.**—Gram-positive; rods 0.8 to 0.9 by 4.0 to 6.1  $\mu$ , occurring singly and in chains. Form central ellipsoidal spores 1.1 by 1.9  $\mu$ . Motile by means of peritrichous flagella. Grows rapidly on agar and slowly with liquefaction on gelatin. Produces no change in litmus milk. Produces indol, does not reduce nitrates, produces acid in lactose, and hydrolyzes starch. Facultatively aerobic. Does not produce ammonia in peptone but fixes 2.8 mgm. of nitrogen in soil.

**CULTURE 217.**—Gram-positive, nonmotile, nonspore-forming; rods 0.45 to 0.6 by 1.2 to 2.4  $\mu$ , occurring singly and in clusters. Grows rapidly on agar and broth. Grows slowly with liquefaction on gelatin. Reduces litmus milk with alkaline reaction. Does not produce indol; does reduce nitrates; does not produce acid on carbohydrate media. Hydrolyzes starch. Aerobic. Fixes 2.8 mgm. of nitrogen in soil.

**CULTURE 219A.**—Gram-positive, nonmotile, nonspore-forming; micrococci 0.6 to 0.9  $\mu$  in diameter. Makes abundant growth on agar, slow growth on gelatin without liquefaction, and no growth on potato. Does not produce indol but reduces nitrates. Does not hydrolyze starch. Aerobic. Produces 1.2 mgm. of ammonia on peptone and fixes 2.8 mgm. of nitrogen in soil.

**CULTURE 221B.**—Gram-positive, motile; rods 0.8 to 0.9 by 3.6 to 5.5  $\mu$ , occurring singly, in pairs, and in short chains. Central ellipsoidal spores 0.8 by 1.8 to 2.2  $\mu$ . Makes abundant growth on ordinary laboratory media. Liquefies gelatin. Peptonizes milk, with an alkaline reaction. Produces indol; reduces nitrates; produces acid on glucose. Hydrolyzes starch. Aerobic. Produces 9.9 mgm. of ammonia in peptone and fixes 2.8 mgm. of nitrogen in soil.

CULTURE 223.—Gram-positive; rods 0.8 to 1.1 by 2.8 to 8.7  $\mu$ . Motile by means of peritrichous flagella. Forms central spores 1.0 to 1.1 by 1.8 to 2.1  $\mu$ . Makes abundant growth on all ordinary laboratory media. Liquefies gelatin, darkens potato. Slowly peptonizes litmus milk with alkaline reaction and the reduction of the litmus. Produces indol, reduces nitrates, produces acid on glucose and sucrose, and hydrolyzes starch. Produces hydrogen sulphide. Aerobic. Produces 25.9 mgm. of ammonia in peptone but does not fix nitrogen.

CULTURE 225C.—Gram-positive; rods 0.8 to 1.8 by 4.5 to 13.7  $\mu$ , occurring in chains. Motile by means of peritrichous flagella. Forms central ellipsoidal spores. Makes rapid growth on agar, gelatin, and broth. Liquefies gelatin. Coagulates milk, with acid reaction. Does not produce indol, reduces nitrates, and produces acid in glucose and lactose. Hydrolyzes starch. Aerobic. Produces 7.1 mgm. of ammonia in peptone and fixes 2.8 mgm. of nitrogen in soil.

CULTURE 226.—Gram-positive, nonmotile; rods 0.8 to 0.9 by 2.7 to 3.2  $\mu$ , occurring singly. Forms central spores 1.1 to 1.6 by 2.1 to 2.8  $\mu$ . Makes rapid and abundant growth on agar and broth and slow growth on gelatin, with liquefaction. Produces acid in milk. Forms indol, hydrolyzes starch, but does not reduce nitrates. Aerobic. Produces 8.5 mgm. of ammonia in peptone and fixes small quantities of nitrogen in soil.

### SUMMARY

This study shows the physiological and morphological properties of 21 microorganisms obtained from a natural-occurring alkali soil which had been partially reclaimed by leaching. Of the 21, 6 were fungi and 15 were bacteria; 13 of the bacteria were bacillus forms and 2 were coccus forms. Eight of the bacilli were motile, 6 formed spores, and 19 of the organisms were Gram-positive. Nineteen liquefied gelatin, 18 hydrolyzed starch, 12 peptonized milk, 9 reduced nitrates, 13 produced indol; 15 fermented glucose, 4 lactose, and 6 sucrose.

Eighteen produced ammonia when grown in peptone. The quantity of ammonia produced in four days ranged from 0.3 mgm. to 26 mgm. Ten of the organisms produced over 5 mgm. of ammonia. Fifteen of the organisms fixed nitrogen when grown in soil to which 1 per cent of mannite had been added. The quantity fixed varied from 1.4 to 7 mgm. in 100 gm. of soil. Three fixed over 4 mgm., and these are probably the organisms, or at least among the organisms, which are causing this soil to gain nitrogen. Two of the organisms decomposed cellulose and one liberated ammonia from urea.

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## THE WATER REQUIREMENT OF CERTAIN CROP PLANTS AND WEEDS IN THE NORTHERN GREAT PLAINS<sup>1</sup>

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### INTRODUCTION

The term "water requirement" has been defined by Briggs and Shantz (1)<sup>3</sup> as the ratio of the weight of water absorbed by a plant during its growth to the weight of dry matter produced, exclusive of the roots.

The experiments reported in this paper were planned in connection with the more extensive investigations of the water requirement of plants under diverse climatic conditions as projected by the authors just cited. The writer's investigations also involved the determination of the water requirement of related strains of alfalfa, millet, and sorgo which he had selected for adaptation to dry-land conditions. These selections were grown in the experiments in order to determine (1) whether the water requirement can be used as a measure of the relative adaptation to conditions of drought of different varieties of the same crop, (2) whether similar varieties or selected progeny strains show appreciable differences in water requirement, and (3) whether the water requirement of crop plants is correlated with yields as determined by plot tests in the field.

These experiments were carried on for seven years, 1912 to 1918, inclusive, at the Belle Fourche Field Station, Newell, S. Dak., and for four years, 1919 to 1922, inclusive, at the United States Northern Great Plains Field Station, Mandan, N. Dak. The experiments at the Belle Fourche station were planned in consultation with T. H. Kearney of the Office of Alkali and Drought Resistant Crops, Bureau of Plant Industry, and H. L. Shantz, formerly of that office. In 1919 the experiments were transferred to Mandan, N. Dak. In 1921 the writer was transferred from the Office of Alkali and Drought Resistant Crops to the Office of Cereal Investigations (now the Office of Cereal Crops and Diseases), but continued the water-requirement investigations at Mandan in 1921 and 1922.<sup>4</sup>

A brief description of the climate, soil, and agricultural conditions in each locality is given under the headings for the respective stations.

<sup>1</sup> Received for publication Sept. 10, 1930; issued February, 1931.

<sup>2</sup> Formerly physiologist, Office of Alkali and Drought Resistant Crops, Bureau of Plant Industry.

<sup>3</sup> Reference is made by number (italics) to Literature Cited, p. 238.

<sup>4</sup> The writer is greatly indebted to Beyer Aune, superintendent of the Belle Fourche Field Station, Office of Western Irrigation Agriculture, for cordial cooperation in furnishing labor during the course of the experiments at Newell, and to J. M. Stephens, superintendent of the United States Northern Great Plains Field Station, Office of Dry Land Agriculture, for equally generous cooperation at Mandan.

## EXPERIMENTAL METHODS

The methods employed were essentially the same as those described by Briggs and Shantz (1). The plants were grown in large galvanized-iron pots having a capacity of about 115 kgm. of soil. The pots were fitted with tight covers sealed at the edges with tape coated with shellac. Holes were punched in the covers, through which the seedling plants emerged, the space surrounding the plants being sealed water-tight with wax. A larger hole in the middle of the cover was used for watering the plants. The cork stopper inserted in this hole was provided with a capillary tube of lead to equalize atmospheric pressure within and without the pot and thus prevent the entrance of water around the plants due to rapid cooling of the pot during showers.

The pot covers were of two types: (1) Those with 21 holes one-half inch in diameter, which were used for wheat, millet, flax, and other small-stemmed plants; and (2) those with 6 holes  $1\frac{1}{2}$  inches in diameter, which were used for alfalfa, sorgo, and the perennial grasses. The grasses having rootstocks were grown fairly successfully by confining the rootstocks to a limited space by means of metal cones placed beneath the covers. The galvanized-iron cones were about 4 inches long,  $1\frac{1}{2}$  inches in diameter at the upper end, and 3 inches in diameter at the lower end. These were set in the soil, the small end of the cone fitting into the hole in the cover, and the seedling grass plants were set in place. The rootstock by this means were forced to grow upward through the holes in the cover rather than to spread laterally in the surface soil just below the cover, as they naturally would do.

The pots were weighed with a spring balance of a capacity of 150 kgm., which was checked frequently against a known weight. The initial weight usually was not taken until the seedling plants had reached a height of 2 or 3 inches, when the plants were sealed in with wax and the weight of the pot recorded. The pots were weighed at frequent intervals, usually every second day in the early growth period and every day when the absorption of water was most rapid. Water was added from calibrated 2-liter flasks. The final weight was taken accurately to 0.1 kgm. immediately after the plants were harvested. The plants were cut off at the surface of the cover, except in the case of alfalfa. The alfalfa plants were clipped carefully so as to leave one or two growing buds at the base of each remaining stem, in order to promote quick revival of growth.

As a protection against birds and possible hail storms the plants were grown within a screened inclosure 20 feet wide, 40 feet long, and 9 feet high. This provided space for 96 pots arranged in four rows running east and west with board walks between. The inclosure was covered with  $\frac{3}{8}$ -inch galvanized-wire mesh supported by iron pipes. The plants were protected from high winds by the board frame (3 feet high) of the inclosure and also by a strip of thin white muslin attached to the screen above the board frame. Briggs and Shantz (1) found that the water requirement of Kubanka wheat grown in pots sunk in trenches in a field of growing wheat was about 10 per cent higher than that of the same variety when grown within the shelter.

The soil used was always surface soil in good tilth, obtained from a plot that had been kept fallow the previous season. In order to

insure adequate fertility a fertilizer was added, composed of 25 parts per million of  $\text{PO}_4$ , 50 parts of  $\text{NO}_3$ , and 33 parts of K, all based on a dry weight of 100 kgm. of soil per pot. The phosphoric acid was applied as sodium phosphate and the nitrogen and potash as potassium nitrate. The fertilizer was dissolved in water and added in three or four portions during the growing season. An application of 2 liters of the dilute solution was followed at once by 2 liters of water to prevent temporary wilting of the foliage.

#### GENERAL CLIMATIC CONDITIONS

The weather records of the Biophysical Laboratory, Bureau of Plant Industry, are available for study in connection with the water-requirement data. These records include daily precipitation; air temperature as recorded by a standardized thermograph; relative humidity; hourly wind velocity; and daily evaporation from the free water surface of a tank 8 feet in diameter and 2 feet deep, the water maintained at ground level, about 4 inches below the edge of the tank.

The general similarity of the climatic conditions at Newell, S. Dak., and at Mandan, N. Dak., during the period of the experiments is shown in Table 1, where the annual and seasonal precipitation and the seasonal evaporation are given for each year of the experiments and as averages for a long period at each station.

More detailed weather data are presented under the results at each station in a discussion of the relation between the seasonal weather conditions and the water requirement of the several crops.

TABLE 1.—*Annual and seasonal (April to August, inclusive) precipitation, and seasonal evaporation from a free water surface, 1912–1918 at Newell, S. Dak., and 1919–1922 at Mandan, N. Dak.*

Place and year	Precipitation		Evapora- tion, seasonal
	Annual	Seasonal	
Newell, S. Dak.:	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>
1912.....	16.09	10.87	34.03
1913.....	12.53	5.94	32.43
1914.....	11.70	7.86	30.92
1915.....	21.02	15.82	23.50
1916.....	13.40	10.03	27.05
1917.....	13.32	9.66	29.51
1918.....	18.31	11.57	28.62
Mean for 20-year period, 1908 to 1927.....	16.24	11.06	30.02
Mandan, N. Dak.:			
1919.....	13.48	8.86	34.24
1920.....	12.69	8.64	30.60
1921.....	15.23	10.09	33.53
1922.....	17.35	9.63	29.03
Mean for 14-year period, 1914 to 1927.....	15.85	11.42	29.20

#### EXPERIMENTS AT NEWELL, S. DAK.

The Belle Fourche Field Station is located near Newell, about 30 miles northeast of the Black Hills, in western South Dakota. The latitude is about  $44^{\circ} 44' \text{ N.}$ , the longitude  $103^{\circ} 26' \text{ W.}$  The altitude is 2,870 feet above sea level. The soil of this section is a heavy clay, locally known as "gumbo" and classified as Pierre clay (12). It consists of about 35 per cent of clay, 43 per cent of silt, and 13 per cent

of very fine sand. When wet, the soil is extremely sticky and takes up water very slowly. As it dries out it shrinks and forms great cracks which enable it to take up water rapidly during quick heavy rains and in irrigation.

As the soil is composed largely of clay and silt, it will hold a large quantity of water. It has a moisture equivalent of about 30 per cent, and its field-carrying capacity is also about 30 per cent, of which about half is available for the use of plants (9). Fortunately, plants do not appear to suffer from drought for some time after the soil begins to crack. It is possible, therefore, to irrigate, and in pot cultures to water the plants, after the soil becomes easily receptive of water and before the plants suffer from drought. This period, however, is not long, perhaps from two to five days in midsummer, when crops are making most rapid growth.

The native vegetation is chiefly of the short-grass type, with western wheatgrass (*Agropyron smithii*), buffalo grass (*Bulbils dactyloides*), and grama grass (*Bouteloua gracilis*) predominating. Western wheatgrass is the typical grass of the gumbo soil of western South Dakota and is very prominent in creek and river valleys where occasional overflow occurs, and elsewhere in wet seasons.

The average annual precipitation at Newell for the 20-year period 1908 to 1927, inclusive, was 16.24 inches and ranged from 6.64 inches in 1911 to 27.37 inches in 1923. The average seasonal precipitation from April to August, inclusive, was 11.06 inches.

A summary of the seasonal (April to September, inclusive) climatic conditions from 1912 to 1918, inclusive, at Newell is given in Table 2.

TABLE 2.—Summary of monthly (April to September, inclusive) climatic conditions, 1912–1918, at Newell, S. Dak.

Year and month	Air temperature					Average wind ve- locity per hour	Precipi- tation	Evapo- ration
	Average daily—			Absolute—				
	Mean	Maxi- mum	Mini- mum	Maxi- mum	Mini- mum			
	° F	° F	° F	° F.	° F.	Miles	Inches	Inches
1912:								
April.....	47	61	34	78	28	9.5	2.32	4.85
May.....	55	66	43	84	32	11.1	2.26	6.12
June.....	66	80	52	101	39	7.6	.29	8.17
July.....	70	83	57	94	40	6.0	3.20	7.98
August.....	68	81	55	95	47	6.9	2.80	6.60
September.....	52	63	41	94	24	7.6	3.49	3.71
Average or total.....	60	72	47	-----	-----	8.1	14.36	37.73
1913.								
April.....	48	62	35	89	24	6.2	.25	4.70
May.....	53	64	42	95	26	5.9	1.98	4.30
June.....	66	79	54	98	45	6.8	3.10	7.05
July.....	70	85	57	101	42	5.8	.35	8.23
August.....	74	89	59	104	45	5.1	.26	8.14
September.....	59	73	46	97	29	4.5	2.38	4.71
Average or total.....	62	75	49	-----	-----	5.7	.832	37.13
1914:								
April.....	43	56	33	78	8	8.2	1.09	3.37
May.....	55	68	44	85	30	7.7	2.22	5.13
June.....	65	80	53	98	44	6.7	2.09	6.71
July.....	76	92	60	104	48	5.0	1.34	8.74
August.....	69	84	53	102	40	5.0	1.12	6.97
September.....	62	79	47	101	35	6.2	.35	4.19
Average or total.....	62	77	48	-----	-----	6.5	8.21	35.11

TABLE 2.—Summary of monthly (April to September, inclusive) climatic conditions, 1912-1918, at Newell, S. Dak.—Continued

Year and month	Air temperature					Average wind velocity per hour	Precipitation	Evaporation
	Average daily—			Absolute—				
	Mean	Maximum	Minimum	Maximum	Minimum			
1915.	° F.	° F.	° F.	° F.	° F.	Miles	Inches	Inches
April.....	52	64	41	83	26	6.6	2.58	4.45
May.....	51	62	42	84	28	7.4	2.32	3.97
June.....	58	70	48	84	33	6.2	4.74	4.61
July.....	64	75	54	88	41	5.0	5.74	5.35
August.....	66	79	53	97	40	4.1	.44	5.11
September.....	56	69	43	100	30	5.9	1.26	3.90
Average or total.....	58	70	47			5.9	17.08	27.45
1916.								
April.....	42	54	30	73	17	7.8	.64	3.69
May.....	52	65	40	92	25	8.7	3.17	5.27
June.....	60	72	49	89	35	7.3	2.19	5.14
July.....	74	90	61	103	49	5.1	2.01	7.52
August.....	67	82	54	96	42	4.4	2.02	5.44
September.....	58	75	41	98	28	6.2	.20	5.43
Average or total.....	59	73	46			6.6	10.23	32.49
1917:								
April.....	40	50	30	74	19	7.8	2.51	2.02
May.....	50	62	38	91	28	5.7	3.71	4.70
June.....	62	76	47	100	36	6.3	.97	6.27
July.....	75	92	58	108	45	5.3	.80	9.54
August.....	67	82	52	100	39	4.2	1.67	6.98
September.....	60	76	45	88	34	5.1	.35	5.31
Average or total.....	59	73	45			5.7	10.01	34.82
1918.								
April.....	41	51	30	76	18	9.2	2.40	3.28
May.....	55	69	42	90	31	6.6	1.60	5.17
June.....	69	83	54	100	33	4.5	1.17	6.55
July.....	69	82	57	100	46	4.2	3.41	6.48
August.....	70	85	55	100	42	2.8	2.99	7.13
September.....	56	69	43	90	26	2.2	3.08	3.95
Average or total.....	60	73	47			4.9	14.65	32.56

It will be seen that the evaporation from a free water surface varied considerably from month to month and, with the exception of that for May, 1915, was greatest during the warm months of June, July, and August. The mean air temperature of the three summer months, June, July, and August, and the evaporation for the same period each year were as follows:

Year.....	1912	1913	1914	1915	1916	1917	1918
Temperature (°F.).....	68	70	70	63	67	68	69
Evaporation(inches).....	22.7	23.4	22.4	15.1	18.1	22.8	20.2

The lowest evaporation was recorded in the cool season of 1915.

As evaporation is a measure of the intensity of the several weather factors—temperature, humidity, solar radiation, and wind velocity—which also affect transpiration in varying degrees, as shown by Briggs and Shantz (3) and others, it is of interest to note the relation between evaporation and the water requirement of the several crops from year to year. For this purpose the evaporation for 5-day periods from April to September, inclusive, is shown in Table 3.



TABLE 3.—Evaporation by 5-day periods from April to September, inclusive, 1912-1918, at Newell, S. Dak.

Period (dates inclusive)	1912	1913	1914	1915	1916	1917	1918
April:	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>
1-5	0.965	0.488	0.767	0.814	0.463	0.220	0.333
6-10	.950	.477	.494	.767	.586	.296	.545
11-15	.629	.382	.736	.720	.702	.368	.839
16-20	.359	1.158	.580	.888	.630	.367	.443
21-25	.744	.992	.424	.351	.658	.591	.551
26-30	1.202	1.208	.368	.914	.650	.176	.567
Total	4.849	4.705	3.369	4.454	3.689	2.018	3.278
May:							
1-5	.710	.513	.841	.284	.851	.425	1.031
6-10	1.122	.716	.881	.761	1.227	1.047	.843
11-15	.866	.717	.602	1.092	.657	1.263	.777
16-20	1.138	.388	.803	.812	.625	.947	.965
21-25	.779	.777	.559	.591	.624	.460	.965
26-31	1.808	1.191	1.447	.430	1.285	.562	.590
Total	6.423	4.302	5.133	3.970	5.269	4.704	5.171
June:							
1-5	1.540	1.373	1.000	.510	.824	.530	.871
6-10	1.077	1.257	1.096	.700	1.106	1.025	1.127
11-15	1.033	1.194	1.738	.421	.569	1.084	1.160
16-20	1.073	.821	1.232	.912	.893	1.314	1.136
21-25	1.715	1.264	1.552	.918	.746	1.099	1.329
26-30	1.737	1.137	1.094	1.151	1.000	1.219	.932
Total	8.175	7.046	6.712	4.612	5.138	6.271	6.555
July:							
1-5	1.287	1.401	1.371	.758	1.336	1.604	1.476
6-10	1.272	1.555	1.224	.806	1.423	1.757	.705
11-15	1.419	1.580	1.797	.897	1.278	1.566	.917
16-20	.873	1.201	1.692	.843	1.052	1.339	.915
21-25	1.349	.963	1.270	.872	1.363	1.475	.997
26-31	1.780	1.535	1.383	1.176	1.067	1.795	1.472
Total	7.980	8.235	8.737	5.352	7.519	9.536	6.482
August:							
1-5	.679	1.240	1.437	.773	1.183	1.293	1.308
6-10	1.085	.945	1.461	.929	.869	.939	.960
11-15	1.241	1.523	1.181	.892	.541	1.054	1.054
16-20	.858	1.456	.865	.768	1.121	1.078	1.120
21-25	1.216	1.345	1.318	.808	.806	1.427	1.331
26-31	1.525	1.635	.704	.943	.918	1.192	1.356
Total	6.604	8.144	6.966	5.113	5.438	6.983	7.129
September:							
1-5	1.167	1.237	.610	.747	1.238	.791	.682
6-10	.903	.902	.543	.818	.879	.907	.718
11-15	.543	.871	.771	.484	.888	.986	.853
16-20	.399	.728	.514	.969	.922	.673	.605
21-25	.339	.568	.804	.639	.851	1.069	.578
26-30	.362	.401	.952	.349	.621	.881	.515
Total	3.713	4.707	4.194	3.956	5.429	5.307	3.951
Total for season	37.744	37.139	35.111	27.457	32.482	34.819	32.566

## WATER REQUIREMENT OF CROP PLANTS

## WHEAT

Kubanka wheat (*Triticum durum*), C. I. 1440,<sup>5</sup> was grown throughout the period of the experiments reported in this paper. As this variety is well adapted to the climatic conditions of the northern Great Plains it was used as a check on the results from year to year and for comparison with other crops. It was grown also in the experiments at Akron, Colo., as reported by Shantz and Piemeisel (11).

<sup>5</sup> Accession number of the Office of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

The water requirement of Kubanka wheat for the seven years 1912 to 1918, at Newell, is shown in Table 4. Its mean water requirement, based on total dry matter, averaged  $431 \pm 5$  for the 7-year period and ranged from  $333 \pm 2$  in the cool and relatively rainy season of 1915 to  $528 \pm 5$  in the warm, dry season of 1914. (Fig. 1.) Based on grain, the mean water requirement averaged  $1,140 \pm 29$  and ranged from  $920 \pm 25$  in 1915 to  $1,279 \pm 23$  in 1914.



FIGURE 1.—Kubanka wheat grown in the water-requirement experiments at Newell, S. Dak. Photographed July 9, 1914

TABLE 4.—Water requirement of Kubanka wheat (*C. I. 1440*), 1912–1918, at Newell, S. Dak.

Year and period of growth	Pot No.	Dry matter	Grain		Water	Water requirement based on—	
						Grain	Dry matter
		Grams	Grams	Per cent	Kgm.		
1912: May 3 to Aug. 13 (102 days)-----	1	110 0	42 4	39	51 50	1, 214	468
	2	151.2	65.8	44	68. 18	1, 038	452
	3	120.0	43.8	37	56. 53	1, 290	471
	4	129.8	53.2	41	61. 57	1, 156	474
	5	120 0	43.0	36	51. 25	1, 192	427
	6	100 7	34.3	34	49. 12	1, 433	488
Mean-----		122 0	47.1			1, 221±36	463±6
1913: Apr. 21 to Aug. 1 (102 days)-----	1	90.0	41.4	46	40. 4	976	449
	2	99.8	46 7	47	42. 3	906	424
	3	89 0	38 8	44	38. 0	980	427
	4	89.5	40 1	44	40. 6	1, 012	454
	5	88 3	39.7	45	38. 4	968	435
	6	85.0	39 3	46	38 1	970	448
Mean-----		90.3	41.0			969±8	440±4
1914: May 1 to July 30 (96 days)-----	1	92 5	39 2	42	49 2	1, 255	532
	2	95.4	41.6	44	49. 8	1, 197	522
	3	98 0	39.3	40	49. 2	1, 252	502
	4	88.9	35 8	40	49. 2	1, 374	553
	5	94.1	39.7	42	48 9	1, 232	520
	6	90.7	35.8	40	48. 9	1, 366	539
Mean-----		93.3	38.6			1, 279±23	528±5

TABLE 4.—*Water requirement of Kubanka wheat (C. I. 1440), 1912-1918, at Newell, S. Dak.—Continued*

Year and period of growth	Pot No	Dry matter	Grain			Water requirement based on—	
			Water			Grain	Dry matter
		Grams	Grams	Per cent	Kgm.		
1915. May 14 to Aug. 25 (103 days).....	1	118.0	44.4	38	39.0	878	330
	2	126.9	50.0	39	40.8	816	322
	3	125.5	46.3	37	41.6	900	331
	4	136.0	44.2	34	44.6	1,008	343
	5	134.8	50.0	37	44.4	888	330
	6	142.6	47.1	33	48.6	1,032	341
Mean.....		129.6	47.0			920±25	333±2
1916 Apr. 28 to July 31 (94 days).....	1	151.9	47.6	31	50.6	1,063	333
	2	172.5	53.1	31	64.2	1,208	372
	3	139.0	43.2	31	48.9	1,132	352
	4	151.3	42.5	28	53.2	1,252	352
	5	128.5	36.4	28	49.8	1,368	388
	6	133.7	45.8	34	41.9	915	313
Mean.....		146.2	44.8			1,156±45	352±7
1917: May 24 to Aug. 18 (86 days).....	1	108.5	42.3	39	53.5	1,265	493
	2	118.7	46.8	39	54.2	1,158	457
	3	102.0	39.7	39	51.0	1,285	500
	4	111.3	41.0	37	51.9	1,266	466
	5	124.5	47.5	38	62.0	1,305	498
	6	116.3	49.4	42	59.0	1,194	507
Mean.....		113.6	44.5			1,246±17	487±6
1918: May 15 to Aug. 14 (91 days).....	1	83.6	26.1	31	37.6	1,440	450
	2	91.9	30.0	33	39.8	1,327	433
	3	112.1	39.6	35	47.6	1,202	424
	4	126.6	45.8	36	50.2	1,100	397
	5	122.2	46.7	38	48.4	1,036	396
	6	120.4	45.7	38	47.0	1,028	390
Mean.....		109.5	39.0			1,189±51	415±8

The close relationship between the water requirement and evaporation from a free water surface during the growth period of the crop may be observed. If the average water requirement for the period is expressed as 100, then the water-requirement determination for each year may be expressed as a percentage or as a relative figure. For comparison, the evaporation may be expressed in the same manner. The relation between the water requirement of Kubanka wheat and evaporation during the 3-month period from June to August, inclusive, is shown in Table 5.

TABLE 5.—*Annual water requirement of Kubanka wheat, and summer evaporation, 1912-1918, Newell, S. Dak.*

Year	Water requirement		Evaporation, June to August	
	Actual	Relative	Actual	Relative
			Inches	
1912.....	463	107	22.7	113
1913.....	436	101	19.6	98
1914.....	528	123	22.4	111
1915.....	333	77	15.1	75
1916.....	352	82	18.1	90
1917.....	487	113	22.8	113
1918.....	415	96	20.2	100
Average.....	431	100	20.1	100

\* Evaporation May to July, inclusive, being the period covered by the crop grown in 1913.

It will be seen that the years 1912, 1914, and 1917 were years of high evaporation and high water requirement, while 1915 and 1916 were years of low evaporation and low water requirement.

Acres yields are influenced or determined by so many factors, as pointed out by Chilcott (4), that a close correlation can not be expected between water requirement and crop yields from year to year. It may be noted, however, that the years of low water requirement, 1915 and 1916, were years of favorable wheat yields, while in 1914 and 1917 the water requirement was high and the yields low. This subject is discussed at greater length on page 226, under the heading "Effect of Season on Water Requirement."

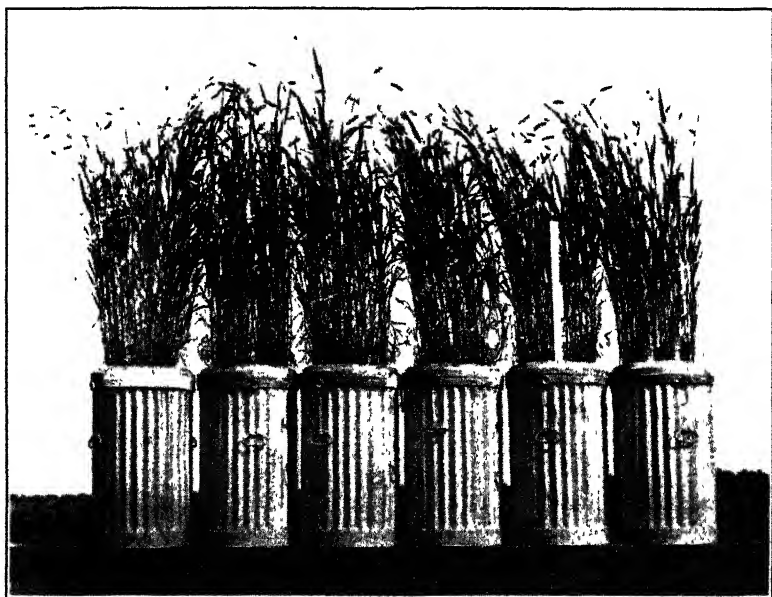


FIGURE 2.—Kursk millet, A. D. I. 3 Photographed August 28, 1915

#### MILLET

Five varieties or strains of millet (*Chaetochloa italica*) were grown for one or more seasons during the course of the experiments at Newell. The strain of Siberian millet, A. D. I. 4-3,<sup>6</sup> and the two strains of Kursk millet, A. D. I. 3 and A. D. I. 13-3, have orange-yellow seeds and belong to the same varietal group. The three strains mentioned were developed by the writer (6) by selection from mixed varieties introduced from Russia by the United States Department of Agriculture. (Fig. 2.) Common millet was grown in 1912, and Gold Mine, a larger type of Common millet, was grown from 1915 to 1917, inclusive. The water requirement of Kursk millet A. D. I. 3 for the seven years 1912 to 1918 is shown in Table 6. The water requirement of Siberian millet, A. D. I. 4-3, is shown in Table 7, and that of the Common, Kursk A. D. I. 13-3, and Gold Mine in Table 8.

<sup>6</sup> Accession number of the former Office of Alkali and Drought Investigations.

The water requirement of the five varieties or strains of millet grown during the 7-year period 1912 to 1918, inclusive, at Newell, S. Dak., is shown in Table 9.

TABLE 6.—*Water requirement of Kursk millet (A. D. I. 3), 1912–1918, at Newell, S. Dak.*

Year and period of growth	Pot No	Dry matter	Grain		Water	Water requirement based on—	
						Grain	Dry matter
		<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>	<i>Kgm.</i>		
1912 June 21 to Sept. 5 (76 days).....	43	259 0	101.4	39	61.3	604	237
	44	223.7	79.9	36	54.3	682	243
	45	227.8	103.3	45	51.2	496	225
	46	226 1	94.5	42	52.3	554	231
	47	240 0	107.2	45	57.8	539	241
	48	242 0	106.0	44	62.1	585	256
Mean.....		236.4	98.7			577±14	239±3
1913 June 10 to Aug. 26 (77 days).....	43	176.8	81.7	46	53.7	657	304
	44	220 0	101.1	46	67.6	669	307
	45	150.3	60.9	41	42.0	690	280
	46	158.8	84.7	45	54.9	648	291
	47	186 0	83.6	45	52.4	627	282
	48	203.7	88.3	43	59.3	672	291
Mean.....		187.6	83.4			661±6	293±3
1914 June 15 to Aug. 31 (77 days).....	37	53.5			13.8		258
	38	44.5			13.1		294
	39	57.8			19.3		334
	40	49.2			16.4		333
	41	49.8			14.4		289
	42	55.2			19.6		355
Mean.....		51.7					311±11
1915 July 1 to Aug. 28 (58 days).....	37	223.7	75.7	34	39.9	527	178
	38	245.2	80.2	33	42.4	529	173
	39	236.3	83.8	35	39.7	474	168
	40	229.2	81.7	36	38.6	472	168
	41	251.0	79.0	32	42.6	539	170
	42	262.0	88.0	34	43.6	495	166
Mean.....		241.2	81.4			506±10	171±1
1916 July 1 to Aug. 28 (58 days).....	13	271.4	63.7	23	63.6	999	234
	14	271.4	73.7	23	62.3	978	230
	15	256.7	67.5	26	59.4	880	231
	16	287.2	70.8	25	66.6	941	232
	17	244.6	55.2	23	57.3	1,038	234
	18	250.1	67.3	27	59.3	881	236
Mean.....		263.6	64.7			953±10	233±1
1917 July 1 to Aug. 31 (61 days).....	13	205.6			57.7		281
	14	168.0			46.5		277
	15	195.5	34.0	17	53.1	1,560	272
	16	174.5	20.6	12	49.0	2,380	281
	17	181.0	21.0	12	49.3	2,350	272
	18	193.8			55.5		286
Mean.....		186.4	25.2			2,096±214	278±2
1918 June 20 to Aug. 19 (60 days).....	31	178.1	31.1	17	43.5	1,398	244
	32	171.6	37.3	22	39.9	1,070	233
	33	177.6	39.3	22	51.4	1,307	289
	34	180.4	49.2	27	42.2	858	234
	35	196.3	30.9	16	43.1	1,395	220
	36	198.7	43.3	22	43.3	1,000	218
Mean.....		183.8	33.5			1,171±74	239±7

TABLE 7.—*Water requirement of Siberian millet (A. D. I. 4-3), 1912-1917, at Newell, S. Dak.*

Year and period of growth	Pot No.	Dry matter	Grain		Water	Water requirement based on—	
						Grain	Dry matter
1912: June 21 to Sept. 5 (76 days) -----		<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>	<i>Kgm.</i>		
	37	252.0	89.8	36	63.3	705	251
	38	245.7	93.0	38	63.3	681	258
	39	274.7	101.6	37	69.0	679	251
	40	290.0	113.4	39	69.6	614	240
	41	286.0	111.6	39	64.5	578	225
	42	263.0	75.1	29	62.5	832	238
Mean -----		268.6	97.4			682±22	244±4
1913. June 10 to Aug. 26 (77 days) -----	37	210.0	80.9	39	68.2	844	325
	38	193.6	69.0	36	63.6	922	328
	39	182.0	48.8	27	60.5	1,240	332
	40	197.8	74.0	37	62.0	838	313
	41	191.2	77.7	41	62.5	805	327
	42	189.0	74.2	39	62.3	840	330
Mean -----		193.9	70.8			915±42	326±2
1914: June 15 to Aug. 31 (77 days) -----	31	75.0			21.3		288
	32	85.8			24.8		289
	33	65.1			18.2		280
	34	90.5			30.1		333
	35	91.3			27.9		306
	36	95.2			30.8		324
Mean -----		83.8					303±7
1915. July 1 to Aug. 30 (60 days) -----	31	251.3	72.5	29	43.4	596	173
	32	244.0	73.1	30	41.2	564	169
	33	247.5	71.5	29	45.9	642	185
	34	244.7	70.6	29	44.0	623	180
	35	258.7	75.3	29	45.8	608	177
	36	253.8	72.5	29	44.7	617	176
Mean -----		250.0	72.6			608±8	177±2
1916. July 1 to Aug. 28 (58 days) -----	7	266.3	52.4	20	61.5	1,173	231
	8	245.7	50.9	21	52.2	1,025	212
	9	255.5	59.2	23	59.4	1,004	232
	10	220.0	43.9	20	49.9	1,137	227
	11	260.7	65.2	24	63.4	972	243
	12	259.7	55.3	21	63.4	1,146	244
Mean -----		251.3	54.5			1,076±29	231±3
1917. July 1 to Aug. 21 (61 days) -----	7	202.6	26.0	13	57.8	2,223	285
	8	223.1	35.0	16	60.9	1,740	273
	9	214.1	30.4	14	58.0	1,907	271
	10	193.0			55.0		285
	11	202.3			54.4		269
	12	198.8			53.8		271
Mean -----		205.7				1,957	275±2

TABLE 8.—*Water requirement of three varieties of millet at Newell, S. Dak.: Common in 1912; Kursk, A. D. I. 13-8, in 1912 and 1913; and Gold Mine, 1915-1917*

Plant and period of growth	Pot No.	Dry matter	Grain		Water	Water requirement based on—	
						Grain	Dry matter
1912: June 21 to Sept. 14 (85 days): Common, A. D. I. 8 -----		<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>	<i>Kgm.</i>		
	31	221.7	80.4	37	65.0	809	293
	32	227.9	96.9	43	69.5	718	305
	33	219.0	83.8	38	71.3	850	325
	34	208.2	85.7	41	65.6	766	315
	35	201.1	82.5	41	62.2	754	309
	36	209.6	84.8	41	63.3	746	302
Mean -----		214.6	85.7			774±14	308±4

TABLE 8.—*Water requirement of three varieties of millet at Newell, S. Dak.: Common in 1912; Kursk, A. D. I. 13-3, in 1912 and 1913; and Gold Mine, 1915-1917—Continued*

Plant and period of growth	Pot No.	Dry matter	Grain		Water	Water requirement based on—	
			Grams	Per cent		Grain	Dry matter
1912 June 21 to Sept. 5 (76 days)		<i>Grams</i>	<i>Grams</i>		<i>Kgm</i>		
Kursk, A. D. I. 13-3	49	238.5	110.4	46	60.7	550	254
	50	233.5	108.7	47	59.9	550	256
	51	240.0	108.0	45	58.3	539	243
	52	252.3	113.2	45	63.2	558	251
	53	247.7	105.0	43	59.2	564	239
	54	264.1	111.7	45	61.5	522	233
Mean		246.0	110.5			547±4	246±3
1913 June 10 to Aug. 26 (77 days)							
Kursk, A. D. I. 13-3	31	188.2	80.9	43	61.1	754	324
	32	196.3	89.0	43	64.7	727	330
	33	183.6	86.0	47	59.6	693	325
	34	216.0	97.0	45	72.1	744	334
	35	177.8	77.2	43	56.7	735	319
	36	177.3	80.1	43	61.0	762	344
Mean		189.9	85.0			736±4	329±3
1915 July 1 to Sept. 1 (62 days)							
Gold Mine	85	211.1		25	38.3		181
	86	220.6	55.0	25	39.4	716	179
	87	208.0	48.5	23	38.9	802	187
	88	239.5	55.5	23	44.3	798	185
	89	210.6	50.0	23	38.2	764	181
	90	227.2	57.8	25	43.2	748	190
Mean		219.5	53.4			766±12	184±2
1916 July 1 to Sept. 1 (62 days)							
Gold Mine	19	305.3	36.9	12	70.0	1,896	229
	20	282.1	34.9	12	67.0	1,918	237
	21	298.1	43.5	15	68.7	1,580	230
	22	328.6	54.2	16	76.2	1,405	234
	23	309.1	44.9	15	72.2	1,607	233
	24	299.3	41.9	14	67.0	1,598	224
Mean		303.8	42.7			1,667±60	231±1
1917: July 1 to Sept. 7 (68 days)							
Gold Mine	19	180.3			50.5		280
	20	221.9	20.0	9	62.5	3,125	282
	21	207.4	19.5	9	57.9	2,970	279
	22	213.0	18.0	8	58.0	3,220	272
	23	208.8			59.7		286
	24	204.0			57.0		280
Mean		205.9				3,105	280±1

TABLE 9.—*Annual water requirement of five varieties of millet, 1912-1918, Newell, S. Dak.*

Plant	Annual water requirement							Average
	1912	1913	1914	1915	1916	1917	1918	
Kursk, A. D. I. 3	239±3	293±3	311±11	171±1	233±1	278±2	239±7	252±4
Siberian, A. D. I. 4-3	244±4	326±2	303±7	177±2	231±3	275±2		259±3
Kursk, A. D. I. 13-3	246±3	329±3						
Common	308±4							
Gold mine				184±2	231±1	280±1		
Average	259±3	316±3	307±9	177±2	232±2	278±2	239±7	

<sup>a</sup> Six-year average.

In 1912 the water requirement of Common millet (308±4) was higher than that of the three other varieties grown. In 1913, Kursk, A. D. I. 3, gave a lower value (239±3) than that of two other varieties grown. Except in these cases there was no significant difference in the water requirement of the varieties tested.

The water requirement of Kursk millet, A. D. I. 3, averaged  $252 \pm 4$  for the 7-year period and ranged from  $171 \pm 1$  in 1915 to  $311 \pm 11$  in 1914. Siberian millet had a water requirement of  $259 \pm 3$  for the 6-year period 1912 to 1917, inclusive.

Based on grain production, Kursk millet, A. D. I. 3, averaged  $994 \pm 55$  and ranged from  $506 \pm 10$  in 1915 to  $2,096 \pm 214$  in 1917. The high figure in 1917 can be explained by the fact that the crop was harvested before the seed was fully mature. In weight of grain per pot Kursk millet weighed more than Kubanka wheat and also had a lower water requirement based on grain.



FIGURE 3.—Dakota Amber sorgho as grown in cultivated rows. Photographed in the field, with a net as background, August 22, 1914

#### SORGO AND SUDAN GRASS

Two varieties of sweet sorghum—Sorgo (*Sorghum vulgare*) and Sudan grass (*S. vulgare* var. *sudanense*)—were grown during the period 1915 to 1918 at Newell. The water requirement of Dakota Amber sorgho (Table 10), based on dry matter, averaged  $252 \pm 3$  and ranged from  $210 \pm 4$  in 1915 to  $284 \pm 3$  in 1918. Seed matured in three of the four seasons, and the water requirement, based on grain, averaged  $1,031 \pm 50$ . (Fig. 3.)

Red Amber sorgho, a later and more leafy type than Dakota Amber, was grown for three years, 1915 to 1917. (Table 11.) Its water requirement, based on dry matter, averaged  $227 \pm 2$ . (Fig. 4.)

Sudan grass (Table 12) was grown for four years, 1915 to 1918. Its water requirement, based on dry matter, ranged from  $272 \pm 2$  in 1915 to  $344 \pm 5$  in 1917 and averaged  $316 \pm 4$ . The yield of seed was recorded only in 1915, when the water requirement, based on grain, was  $2,000 \pm 78$ .





FIGURE 4.—Sudan grass (at left) and Red Amber sorgho grown in the water-requirement experiments at Newell, S. Dak. Photographed September 8, 1917

TABLE 10.—Water requirement of Dakota Amber sorgho, 1915–1918, at Newell, S. Dak.

Year and period of growth	Pot No.	Dry matter	Grain		Water	Water requirement based on—	
						Grain	Dry matter
		<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>	<i>Kgm.</i>		
1915: July 1 to Sept. 28 (89 days)-----	43	156.7	43.5	28	36.4	837	232
	44	223.8	51.6	23	49.9	967	223
	45	267.7	63.3	24	51.9	820	194
	46	272.4	90.7	33	52.7	581	194
	47	241.8	45.8	19	50.6	1,105	209
	48	253.7	66.3	26	52.3	789	206
Mean-----		236.0	60.2			850±46	210±4
1916: July 1 to Sept. 11 (72 days)-----	31	225.3	65.1	29	57.1	878	253
	32	287.3	76.8	27	67.6	880	235
	33	274.7	70.5	26	65.3	926	238
	34	302.6	87.4	29	71.5	818	236
	35	253.5	57.6	23	58.6	1,017	231
	36	285.7	79.7	28	68.1	855	239
Mean-----		271.5	72.9			896±19	239±2
1917: July 1 to Sept. 8 (68 days)-----	31	223.0	50.0	22	60.2	1,204	270
	32	202.5	37.0	18	54.8	1,480	271
	33	226.3	62.5	28	63.9	1,022	282
	34	227.0	37.0	16	63.7	1,720	280
	35	261.7	55.0	21	72.5	1,318	277
	36	167.5			44.8		268
Mean-----		218.0	48.3			1,349±85	275±2
1918: June 25 to Aug. 30 (66 days)-----	85	171.3			47.8		279
	86	134.9			37.2		276
	87	109.2			31.8		291
	88	97.6			27.4		281
	89	137.7			38.0		276
	90	117.4			35.2		300
Mean-----		128.0					284±3

TABLE 11.—Water requirement of Red Amber sorgo, 1915-1917, at Newell, S. Dak.

Year and period of growth	Pot No.	Dry matter	Water	Water requirement based on dry matter
1915: July 1 to Sept 28 (89 days).....		<i>Grams</i>	<i>Kgm.</i>	
	49	260.4	55.2	209
	50	297.2	59.3	199
	51	320.4	64.2	198
	52	322.5	65.6	203
	53	257.4	53.8	209
	54	312.0	63.3	203
Mean.....		295.0		204±1
1916: July 1 to Sept 11 (72 days).....	37	359.3	85.3	237
	38	392.0	90.5	231
	39	317.1	76.1	240
	40	377.3	83.4	221
	41	365.8	86.9	238
	42	363.6	80.8	222
Mean.....		362.5		232±3
1917: July 1 to Sept. 8 (68 days).....	37	274.0	66.5	243
	38	334.1	82.1	246
	39	292.6	72.1	248
	40	345.0	84.4	245
	41	299.1	75.9	253
	42	314.7	75.0	239
Mean.....		309.7		246±1

TABLE 12.—Water requirement of Sudan grass, 1915-1918, at Newell, S. Dak.

Year and period of growth	Pot No.	Dry matter	Grain	Water	Water requirement based on—	
					Grain	Dry matter
1915: July 1 to Sept 28 (89 days).....		<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>	<i>Kgm.</i>	
	25	190.7	28.7	15	51.6	1,798
	26	208.7	28.4	14	55.3	1,946
	27	191.9	22.7	12	52.2	2,300
	28	165.0	24.7	15	45.5	1,843
	29	213.2	24.7	12	57.2	2,315
	30	189.3	29.7	16	53.3	1,795
Mean.....		193.1	26.5			2,000±78
1916: July 1 to Sept. 11 (72 days).....	25	314.2			101.3	322
	26	258.2			87.1	337
	27	313.0			93.0	297
	28	281.7			90.3	321
	29	280.5			85.3	304
	30	277.3			84.9	306
Mean.....		287.5				314±5
1917: July 1 to Sept. 8 (68 days).....	25	203.8			70.2	345
	26	212.3			75.5	355
	27	195.7			71.0	363
	28	199.8			63.1	316
	29	208.7			69.9	335
	30	202.1			71.2	352
Mean.....		203.7				344±5
1918: June 25 to Aug. 30 (66 days).....	25	166.8			54.7	328
	26	166.2			55.5	334
	27	154.7			53.9	348
	28	173.7			55.6	320
	29	171.0			55.6	325
	30	164.0			55.8	340
Mean.....		166.1				333±3

The average water requirement of Red Amber sorgo ( $227 \pm 2$ ) was the same as Kursk millet (Table 6) for the three years 1915 to 1917, inclusive. Dakota Amber sorgo required somewhat more water ( $252 \pm 3$ ) than Kursk millet ( $230 \pm 3$ ) per unit of dry matter produced during the 4-year period 1915 to 1918, inclusive. Sudan grass required about 37 per cent more water than millet and 25 per cent more water than Dakota Amber sorgo per unit of dry matter produced.

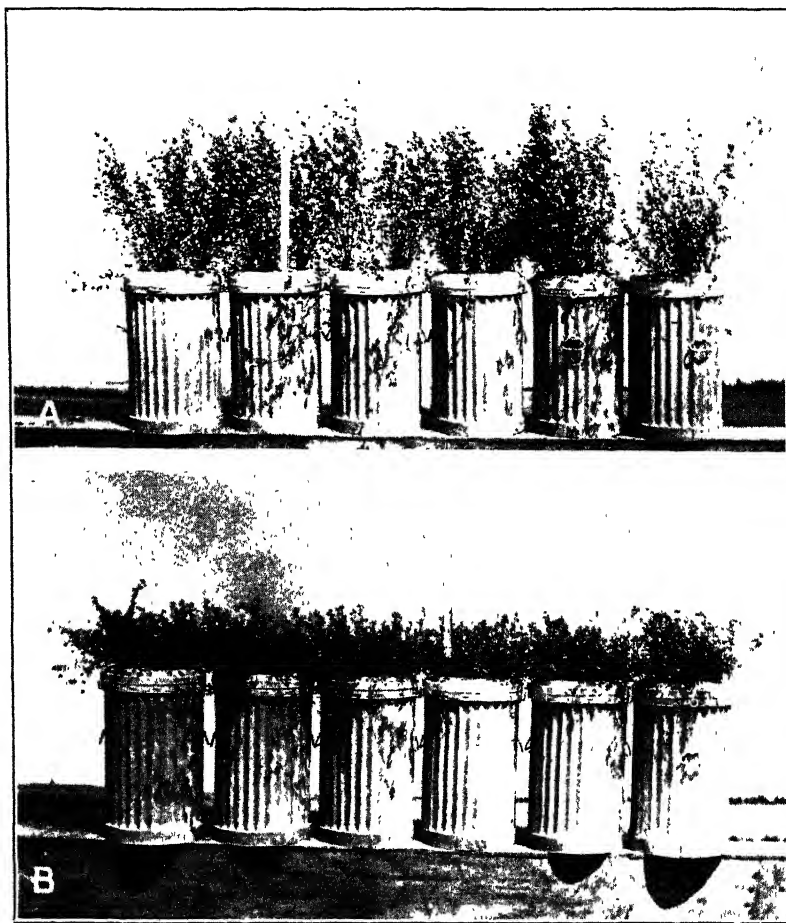


FIGURE 5.—A, Second crop of Turkistan alfalfa, A. D. I. F12-36, overwintered from the planting made in 1914; B, yellow-flowered alfalfa, *Medicago falcata*. Both photographed just before harvesting the second crop of the season, August 4, 1915, Newell, S. Dak.

At Akron, Colo., Shantz and Piemeisel (11) found that during the years 1911 to 1917, inclusive, the water requirement of Kursk millet was the same as that of Minnesota Amber sorgo ( $274 \pm 3$ ) and that the water requirement of Sudan grass ( $380 \pm 3$ ) was nearly 39 per cent higher than that of millet and sorgo.

## ALFALFA

Nine varieties and strains of common alfalfa (*Medicago sativa*) and the yellow-flowered alfalfa (*M. falcata*) were grown in the water-requirement experiments at Newell, S. Dak., during the 7-year period 1912 to 1918, inclusive. Generally two or three crops were obtained each year, the plants being cut when in the early flowering stage, that is, in about the proper stage of growth for hay. The yield was not large, the total weight of dry matter ranging from about 50 to 200 gm. per pot. (Fig. 5.)

The water requirement of Grimm alfalfa, A. D. I. E-23, is shown in Table 13. The value shown in the last column represents a mean of six pots, except as otherwise noted, determined individually in each experiment.

The water requirement of the combined crop averaged  $814 \pm 14$  and ranged from  $696 \pm 18$  in 1915 to  $1,038 \pm 25$  in 1914. In nearly every case the early-summer crop was produced with a lower water requirement than the midsummer crop and the midsummer more economically than the late-summer crop. This difference was marked in 1913, when the plants overwintered from the planting in 1912.

TABLE 13.—Water requirement of Grimm alfalfa (A. D. I. E-23), 1912-1918, at Newell, S. Dak.

Year and crop	Period of growth	Days of growth	Pot Nos. (inclusive)	Dry matter (average of 6 pots)	Water transpired (average of 6 pots)	Water requirement based on dry weight
1912 (from seed).		Number		Grams	Kgm	
Midsummer.....	June 22 to Aug. 8.....	47	7-12	38.4	26.9	$699 \pm 15$
Late summer.....	Aug. 8 to Sept. 24.....	47	7-12	38.0	29.1	$772 \pm 18$
Combined crop.....	June 22 to Sept. 24.....	94	7-12	76.8	55.9	$735 \pm 15$
1913 (planted in 1912)						
Early summer.....	Apr. 30 to June 30.....	61	7-12	36.6	17.2	$469 \pm 6$
Midsummer.....	June 30 to Aug. 6.....	37	7-12	27.0	21.9	$817 \pm 13$
Late summer.....	Aug. 6 to Sept. 18.....	43	7-12	20.3	22.6	$1,115 \pm 16$
Combined crop.....	Apr. 30 to Sept. 18.....	141	7-12	83.8	61.7	$735 \pm 8$
1914 (from seed).						
Midsummer.....	June 25 to Aug. 5.....	41	55-60	22.6	19.8	$877 \pm 25$
Late summer.....	Aug. 5 to Oct. 1.....	57	55-60	32.7	37.3	$1,159 \pm 35$
Combined crop.....	June 25 to Oct. 1.....	98	55-60	55.2	57.1	$1,038 \pm 25$
1914 (one pot, planted in 1912)*						
Early summer.....	May 26 to June 25.....	30	25	43.8	23.6	539
Mid to late summer.....	June 25 to Sept. 2.....	69	25	43.4	34.7	800
Combined crop.....	May 26 to Sept. 2.....	99	25	87.2	58.3	669
1915 (from seed)						
Midsummer.....	July 1 to Aug. 5.....	35	73-78	32.0	14.0	$442 \pm 14$
Late summer.....	Aug. 5 to Sept. 20.....	46	73-78	38.6	48.5	$836 \pm 19$
Combined crop.....	July 1 to Sept. 20.....	81	73-78	90.7	62.5	$696 \pm 18$
1915 (planted in 1914)*						
Early summer.....	Apr. 27 to June 30.....	64	55-60	57.4	24.3	$424 \pm 9$
Midsummer.....	June 30 to Aug. 5.....	36	55-60	66.5	27.3	$413 \pm 7$
Late summer.....	Aug. 5 to Sept. 20.....	46	55-60	77.1	48.3	$630 \pm 18$
Combined crop.....	Apr. 27 to Sept. 30.....	146	55-60	200.9	99.9	$499 \pm 7$
1916 (from seed):						
Midsummer.....	July 1 to July 26.....	25	85-90	34.8	21.8	$622 \pm 11$
Late summer.....	July 26 to Sept. 1.....	37	85-90	49.6	36.6	$738 \pm 8$
Autumn.....	Sept. 1 to Oct. 2.....	31	85-90	29.1	18.0	$621 \pm 12$
Combined crop.....	July 1 to Oct. 2.....	93	85-90	113.4	76.4	$673 \pm 8$

TABLE 13.—*Water requirements of Grimm alfalfa (A. D. I. E.-23). 1912-1918 at Newell, S. Dak.—Continued*

Year and crop	Period of growth	Days of growth	Pot Nos. (inclusive)	Dry matter (average of 6 pots)	Water transpired (average of 6 pots)	Water requirement based on dry weight
1917 (from seed):		<i>Number</i>		<i>Grams</i>	<i>Kgm.</i>	
Midsummer.....	July 1 to July 31.....	30	85-90	32 6	26.2	811±16
Late summer.....	July 31 to Sept 19.....	50	85-90	78 3	69 5	892±18
Combined crop.....	July 1 to Sept. 19.....	80	85-90	110 8	95 8	866±16
1918 (from seed):						
Midsummer.....	June 25 to Aug. 19.....	55	73-78	31 1	30 5	985±21
Late summer.....	Aug 19 to Oct 2.....	44	73-78	20.4	18 4	910±21
Combined crop.....	June 25 to Oct. 2.....	99	73-78	51.4	49 0	955±10
1918 (from seed):						
Crop grown for seed.....	.....do.....	99	79-84	44 2	61 9	1,399±14

In Table 14 is presented the water requirement of eight varieties and strains of alfalfa grown in the experiments at Newell, S. Dak. Five of these are progeny strains of the second, third, or fourth generation, selected by the writer for the purpose of improving the forage and seed-producing characters and for adaptation to drought.

The water requirement of the varieties grown for two or more years, based on the total annual crop, in comparison with Grimm, A. D. I. E-23, is shown in Table 15.

TABLE 14.—*Water requirement of varieties and selected strains of alfalfa, 1912-1918, at Newell, S. Dak.*

Variety, year, and crop	Period of growth	Days of growth	Pot Nos. (inclusive)	Dry matter (average of 6 pots)	Water transpired (average of 6 pots)	Water requirement based on dry weight
Grimm, A. D. I. E-23-20-52:		<i>Number</i>		<i>Grams</i>	<i>Kgm.</i>	
1912 (from seed)—						
Midsummer.....	June 22 to Aug. 8.....	47	19-24	39.1	25.3	646±10
Late summer.....	Aug. 8 to Sept 24.....	47	19-24	44.1	27 7	630±8
Combined crop.....	June 22 to Sept 24.....	94	19-24	83 2	53.0	638±5
1913 (planted in 1912)—						
Early summer.....	Apr. 30 to June 30.....	61	19-24	25.5	11.3	438±11
Midsummer.....	June 30 to Aug. 6.....	37	19-24	22.4	19.4	872±19
Late summer.....	Aug 6 to Sept. 18.....	43	19-24	21 0	22.1	1,081±47
Combined crop.....	Apr. 30 to Sept 18.....	141	19-24	63.9	52.8	766±12
Grimm, A. D. I. E-2-33-72:						
1912 (from seed)—						
Midsummer.....	June 22 to Aug. 8.....	47	13-18	31.5	23.9	755±19
Late summer.....	Aug. 8 to Sept. 24.....	47	13-18	37.7	26.8	712±9
Combined crop.....	June 22 to Sept. 24.....	94	13-18	69.2	50.7	731±12
1913 (planted in 1912)—						
Early summer.....	Apr. 30 to June 30.....	61	13-18	36.8	17.7	478±9
Midsummer.....	June 30 to Aug. 6.....	37	13-18	24.3	22.1	915±26
Late summer.....	Aug. 6 to Sept. 18.....	43	13-18	22.7	25.1	1,108±6
Combined crop.....	Apr. 30 to Sept. 18.....	141	13-18	83 8	64.9	775±9

TABLE 14.—Water requirement of varieties and selected strains of alfalfa, 1912-1918, at Newell, S. Dak.—Continued

Variety, year, and crop	Period of growth	Days of growth	Pot Nos. (inclusive)	Dry matter (average of 6 pots)	Water transpired (average of 6 pots)	Water requirement based on dry weight
<b>Grimm, A. D. I. E-5-30:</b>						
1914 (from seed)—		Number		Grams	Kgm.	
Midsummer	June 4 to Aug. 5	62	61-66	10.3	10.2	993±46
Late summer	Aug. 5 to Oct. 1	57	61-66	22.9	23.2	1,018±18
Combined crop	June 4 to Oct. 1	119	61-66	33.4	33.4	1,005±24
<b>1915 (planted in 1914)—</b>						
Early summer	Apr. 27 to June 30	64	61-66	54.0	25.0	464±8
Midsummer	June 30 to Aug. 5	36	61-66	62.1	24.8	400±4
Late summer	Aug. 5 to Sept. 20	46	61-66	75.8	46.3	611±13
Combined crop	Apr. 27 to Sept. 20	146	61-66	191.9	96.0	501±6
<b>1916 (from seed)—</b>						
Midsummer	July 1 to July 26	25	67-72	29.3	18.5	643±8
Late summer	July 26 to Sept. 1	37	67-72	40.3	32.0	794±14
Autumn	Sept. 1 to Oct. 2	31	67-72	24.5	17.2	697±20
Combined crop	July 1 to Oct. 2	93	67-72	94.0	67.6	718±11
<b>1917 (from seed)—</b>						
Midsummer	July 1 to July 31	30	73-78	25.9	21.5	833±9
Late summer	July 31 to Sept. 19	50	73-78	69.4	64.7	937±20
Combined crop	July 1 to Sept. 19	80	73-78	95.2	86.2	908±16
<b>1918 (from seed)—</b>						
Midsummer	June 25 to Aug. 19	55	55-60	22.6	24.2	1,067±40
Late summer	Aug. 19 to Oct. 2	44	55-60	18.2	17.0	941±8
Combined crop	June 25 to Oct. 2	99	55-60	40.7	41.2	1,023±24
<b>Baltic, A. D. I. H-4-58:</b>						
1914 (from seed)—						
Midsummer	June 4 to Aug. 5	62	19-24	9.8	10.6	1,060±37
Late summer	Aug. 5 to Oct. 1	57	19-24	20.2	21.3	1,039±31
Combined crop	June 4 to Oct. 1	119	19-24	30.0	31.8	1,066±25
<b>1915 (planted 1914)—</b>						
Early summer	Apr. 27 to June 30	64	19-24	48.6	24.9	515±11
Midsummer	June 30 to Aug. 5	36	19-24	55.8	24.9	448±9
Late summer	Aug. 5 to Sept. 20	46	19-24	61.4	41.8	683±12
Combined crop	Apr. 27 to Sept. 20	146	19-24	165.8	91.6	554±10
<b>1916 (from seed)—</b>						
Midsummer	July 1 to July 26	25	49-54	29.9	19.7	668±15
Late summer	July 26 to Sept. 1	37	49-54	42.5	32.1	756±17
Autumn	Sept. 1 to Oct. 2	31	49-54	26.3	17.3	656±4
Combined crop	July 1 to Oct. 2	93	49-54	98.7	69.1	700±13
<b>1917 (from seed)—</b>						
Midsummer	July 1 to July 31	30	55-60	33.3	27.3	824±14
Late summer	July 31 to Sept. 19	50	55-60	78.0	70.6	907±11
Combined crop	July 1 to Sept. 19	80	55-60	111.2	97.9	881±9
<b>1918 (from seed)—</b>						
Midsummer	June 25 to Aug. 19	55	37-42	37.8	34.1	910±27
Late summer	Aug. 19 to Oct. 2	44	37-42	23.2	18.6	807±15
Combined crop	June 25 to Oct. 2	99	37-42	61.0	52.7	871±15
<b>Turkestan, A.D.I. F-12-36:</b>						
1914 (from seed)—						
Midsummer	June 4 to Aug. 5	62	7-12	29.9	26.2	1,214±14
Late summer	Aug. 5 to Oct. 1	57	7-12	28.8	34.7	1,207±18
Combined crop	June 4 to Oct. 1	119	7-12	58.7	70.9	1,210±16

TABLE 14.—*Water requirement of varieties and selected strains of alfalfa, 1912-1918, at Newell, S. Dak.—Continued*

Variety, year, and crop	Period of growth	Days of growth	Pot Nos (inclusive)	Dry matter (average of 6 pots)	Water transpired (average of 6 pots)	Water requirement based on dry weight
<b>Turkestan, A.D.I. F-12-36.—Con</b>						
1915 (from seed)—		Number		Grams	Kgm.	
Midsummer.....	July 1 to Aug. 5.....	35	67-72	33.7	14.4	426±4
Late summer.....	Aug. 5 to Sept. 20.....	46	67-72	51.2	44.9	881±11
Combined crop.....	July 1 to Sept. 20.....	51	67-72	84.9	59.3	695±12
<b>1915 (planted in 1914)—</b>						
Early summer.....	Apr. 27 to June 30.....	64	7-12	70.4	30.2	431±5
Midsummer.....	June 30 to Aug. 5.....	36	7-12	70.2	29.8	425±3
Late summer.....	Aug. 5 to Sept. 20.....	46	7-12	74.6	48.5	651±7
Combined crop.....	Apr. 27 to Sept. 20.....	146	7-12	215.2	108.5	505±3
<b>1916 (from seed)—</b>						
Midsummer.....	July 1 to July 26.....	25	61-66	29.7	17.9	604±6
Late summer.....	July 26 to Sept. 1.....	37	61-66	41.0	29.0	711±17
Autumn.....	Sept. 1 to Oct. 2.....	31	61-66	22.5	14.4	642±19
Combined crop.....	July 1 to Oct. 2.....	93	61-66	93.2	61.3	659±9
<b>1917 (from seed)—</b>						
Midsummer.....	July 1 to July 31.....	30	61-66	32.7	27.7	847±10
Late summer.....	July 31 to Sept. 19.....	50	61-66	74.9	68.7	917±11
Combined crop.....	July 1 to Sept. 19.....	80	61-66	107.7	96.4	896±9
<b>1918 (from seed)—</b>						
Midsummer.....	June 25 to Aug. 19.....	55	61-66	26.5	25.5	961±24
Late summer.....	Aug. 19 to Oct. 2.....	44	61-66	19.1	17.3	908±23
Combined crop.....	June 25 to Oct. 2.....	99	61-66	45.6	42.7	937±23
<b>Grimm, A.D.I. 81.</b>						
1915 (from seed)—						
Midsummer.....	July 1 to Aug. 5.....	35	79-84	36.3	14.6	400±9
Late summer.....	Aug. 5 to Sept. 20.....	46	79-84	59.7	49.1	828±10
Combined crop.....	July 1 to Sept. 20.....	81	79-84	96.0	63.7	667±7
<b>1916 (from seed)—</b>						
Midsummer.....	July 1 to July 26.....	25	79-84	24.4	16.8	697±15
Late summer.....	July 26 to Sept. 1.....	37	79-84	40.2	29.4	735±28
Autumn.....	Sept. 1 to Oct. 2.....	31	79-84	23.7	16.3	696±30
Combined crop.....	July 1 to Oct. 2.....	93	79-84	88.4	62.5	713±20
<b>1917 (from seed)—</b>						
Midsummer.....	July 1 to July 31.....	30	79-84	24.5	22.6	940±25
Late summer.....	July 31 to Sept. 19.....	50	79-84	67.3	63.0	939±15
Combined crop.....	July 1 to Sept. 19.....	80	79-84	91.8	85.6	938±17
<b>Grimm, A.D.I. 62:</b>						
1916 (from seed)—						
Midsummer.....	July 1 to July 26.....	25	55-60	27.9	17.6	635±14
Late summer.....	July 26 to Sept. 1.....	37	55-60	36.4	27.3	752±9
Autumn.....	Sept. 1 to Oct. 2.....	31	55-60	21.1	14.9	707±19
Combined crop.....	July 1 to Oct. 2.....	93	55-60	85.4	59.7	701±9
<b>Common (Kansas grown).</b>						
1916 (from seed)—						
Midsummer.....	July 1 to July 26.....	25	73-78	36.4	21.5	504±14
Late summer.....	July 26 to Sept. 1.....	37	73-78	47.0	32.2	690±20
Autumn.....	Sept. 1 to Oct. 2.....	31	73-78	27.8	16.0	581±15
Combined crop.....	July 1 to Oct. 2.....	93	73-78	111.2	69.7	619±9

TABLE 15.—Annual water requirement of seven varieties of alfalfa for two or more years, 1912–1918, at Newell, S. Dak.

Plant	Annual water requirement							
	1912	1913	1914	1915	1916	1917	1918	Average
Grimm, A. D. I. E-23	735±15	<sup>a</sup> 735±8						<sup>b</sup> 735±11
Grimm, A. D. I. E-23-20-52	638±8	<sup>a</sup> 766±12						<sup>b</sup> 702±8
Grimm, A. D. I. E-2-33-72	731±12	<sup>a</sup> 775±9						<sup>b</sup> 753±10
Grimm, A. D. I. E-23			1,083±25	<sup>a</sup> 499±7	673±8	860±16	955±10	<sup>b</sup> 806±13
Grimm, A. D. I. E-5-30			1,005±24	<sup>a</sup> 501±6	718±11	908±16	1,023±24	<sup>b</sup> 831±16
Baltic, A. D. I. H-4-58			1,066±25	<sup>a</sup> 654±10	700±13	881±9	871±15	<sup>b</sup> 814±14
Turkestan, A. D. I. F-12-36			1,210±16	<sup>a</sup> 505±3	659±9	896±9	937±23	<sup>b</sup> 841±12

<sup>a</sup> Plants overwintered.<sup>b</sup> 2-year average.<sup>c</sup> 5-year average

In the 2-year period 1912 and 1913, Grimm, A. D. I. E-23-20-52 had a water requirement of  $702 \pm 8$  as compared with  $735 \pm 11$  for A. D. I. E-23 and  $753 \pm 10$  for A. D. I. E-2-33-72. These differences are hardly significant. For the 5-year period 1914–1918, Grimm, A. D. I. E-23, had the lowest water requirement ( $806 \pm 13$ ) of the four varieties grown, but again the differences probably are not significant when the probable error is taken into account.

Common alfalfa, Kansas seed, was grown in 1916. (Table 14.) Its water requirement was  $619 \pm 9$  as compared with  $673 \pm 8$  for Grimm A. D. I. E-23.

In purchasing seed for planting, many farmers prefer seeds grown on dry land or under natural conditions of rainfall to those grown under irrigation. In order to determine whether irrigation has any immediate effect on the water requirement of alfalfa, the writer obtained seed of Grimm alfalfa, A. D. I. 81, which had been grown under irrigation at Rocky Ford, Colo., for three years and through two seed generations. This was grown in the water-requirement experiments for three years, 1915 to 1917, inclusive. (Table 14.) The water requirement of this strain, A. D. I. 81, averaged  $773 \pm 15$  as compared with  $745 \pm 14$  for Grimm, A. D. I. E-23 (Table 13), which had been grown continuously under dry-land conditions. The difference probably is not significant when the high probable error is considered. The water requirement of A. D. I. 81 was lower than that of A. D. I. E-23 in the cool season of 1915 but higher in the warmer seasons of 1916 and 1917.

## YELLOW-FLOWERED ALFALFA

Yellow-flowered alfalfa (*Medicago falcata*) was grown for four years, 1912 to 1915, inclusive. (Table 16.) This species grows slowly during the first year from seed, but makes a rapid growth for the first crop the second season, with an economical use of water. It is slow to recover after cutting, and therefore the late summer crop shows a higher water requirement. (Fig. 5, B.) The species is very winter hardy, and it was possible to carry the plants over winter in the pots without winterkilling.

The water requirement of *Medicago falcata* averaged  $617 \pm 13$  during the four years 1912 to 1915, as compared with  $660 \pm 10$  for Grimm A. D. I. E-23 (*M. sativa*). (Table 13.)



TABLE 16.—Water requirement of yellow-flowered alfalfa (*S. P. I.*<sup>a</sup> 28070) 1912-1915, at Newell, S. Dak.

Year and crop	Period of growth	Days of growth	Pot Nos. (inclusive)	Dry matter (average)	Water transpired (average)	Water requirement based on dry matter
1912 (from seed):		Number		Grams	Kgm	
Midsummer.....	July 3 to Aug. 8.....	36	25-30	21.6	13.0	624±15
Late summer.....	Aug. 8 to Sept. 24.....	47	25-30	15.0	10.7	736±18
Combined crop.....	July 3 to Sept. 24.....	83	25-30	36.6	23.7	662±12
1913 (planted in 1912):						
Early summer.....	Apr. 30 to June 30.....	61	25-30	53.0	18.2	340±7
Midsummer.....	June 30 to Aug. 6.....	37	25-30	27.5	19.6	713±11
Late summer.....	Aug. 6 to Sept. 18.....	43	25-30	15.7	18.6	1,220±52
Combined crop.....	Apr. 30 to Sept. 18.....	141	25-30	96.2	56.4	579±12
1914 (planted in 1912):						
Early summer.....	May 7 to June 24.....	48	26-30	37.1	17.4	503±38
Mid to late summer.....	June 24 to Sept. 2.....	70	26-30	29.6	27.6	930±26
Combined crop.....	May 7 to Sept. 2.....	118	26-30	66.7	45.0	687±20
1914 (from seed):						
Midsummer.....	June 25 to Aug. 5.....	41	13-18	11.3	13.7	1,225±26
Late summer.....	Aug. 5 to Oct. 1.....	57	13-18	9.4	17.2	1,837±34
Combined crop.....	June 25 to Oct. 1.....	98	13-18	20.7	30.9	1,500±20
1915 (planted in 1914):						
Early summer.....	Apr. 27 to June 30.....	64	13-18	58.3	23.0	399±8
Midsummer.....	June 30 to Aug. 5.....	36	13-18	36.7	15.5	424±4
Late summer.....	Aug. 5 to Sept. 20.....	46	13-18	22.4	24.7	1,108±20
Combined crop.....	Apr. 27 to Sept. 20.....	146	13-18	117.4	62.2	541±9

<sup>a</sup> Accession number of the Office of Foreign Plant Introduction (formerly Seed and Plant Introduction).

## BROMEGRASS AND WHEATGRASSES

Three perennial grasses, brome grass (*Bromus inermis*), crested wheatgrass (*Agropyron cristatum*), and western wheatgrass (*A. smithii*) were grown for two or more years in the water-requirement experiments at Newell. As mentioned under "Experimental Methods," the stems and rhizomes of the perennial grasses were forced to grow through the holes in the metal covers by means of a cone-shaped funnel placed around the crown of each plant below the cover. Two cuttings were made each year except in 1913, when only one crop of western wheatgrass was obtained. In Table 17 the water requirement of each species is shown.

TABLE 17.—*Water requirement of brome grass (S. P. I. 29880), of crested wheatgrass (S. P. I. 19537), and of western wheatgrass, 1913-1918, at Newell, S. Dak.*

Species, year, and crop	Period of growth	Days of growth	Pot Nos. (inclusive)	Dry matter (average)	Water transpired (average)	Water requirement based on dry matter
Brome grass		Number		Grams	Kgm.	
1913 (from seed)—						
Midsummer.....	May 24 to July 18.....	55	61-66	15.8	15.9	1,025±27
Late summer.....	July 18 to Sept. 22.....	66	61-66	33.9	26.5	791±30
Combined crop.....	May 24 to Sept. 22.....	121	61-66	49.7	42.4	852±26
1916 (from seed)—						
Midsummer.....	July 1 to Aug. 28.....	58	43-48	60.9	40.1	661±15
Late summer.....	Aug. 28 to Oct. 2.....	35	43-48	18.7	16.2	871±30
Combined crop.....	July 1 to Oct. 2.....	93	43-48	79.6	56.3	708±13
1917 (planted in 1916)—						
Midsummer.....	June 18 to July 30.....	42	43-48	35.2	32.7	939±22
Late summer.....	July 30 to Sept. 19.....	51	43-48	26.2	23.3	909±49
Combined crop.....	June 18 to Sept. 19.....	93	43-48	61.4	56.0	921±33
1918 (from seed)—						
Midsummer.....	June 12 to Aug. 19.....	68	43-48	30.4	21.4	704±16
Late summer.....	Aug. 19 to Oct. 2.....	44	43-48	16.6	10.7	645±14
Combined crop.....	June 12 to Oct. 2.....	112	43-48	47.0	32.1	684±14
Crested wheatgrass:						
1913 (from seed)—						
Midsummer.....	May 24 to July 18.....	55	49-54	25.8	16.3	633±9
Late summer.....	July 18 to Sept. 22.....	66	49-54	32.2	34.7	1,083±32
Combined crop.....	May 24 to Sept. 22.....	121	49-54	58.0	51.0	880±10
1914 (planted in 1913, 3 pots only)—						
Early summer.....	May 21 to June 24.....	34	51-53	24.7	10.6	422±13
Midsummer to late summer.....	June 24 to Sept. 30.....	98	51-53	24.8	39.9	1,705±195
Combined crop.....	May 21 to Sept. 30.....	132	51-53	49.5	50.5	1,024±34
Western wheatgrass:						
1913 (from seed)—						
Summer (one crop only).....	May 24 to Sept. 22.....	121	55-60	28.6	35.9	1,247±31
1914 (planted in 1913, 1 pot only)—						
Early summer.....	May 21 to June 24.....	34	54	20.0	11.0	550
Midsummer to late summer.....	June 24 to Sept. 30.....	98	54	18.1	43.1	2,380
Combined crop.....	May 21 to Sept. 30.....	132	54	38.1	54.1	1,420

The water requirement of brome grass averaged  $791 \pm 21$  for the four years 1913 and 1916 to 1918, inclusive. Its lowest value was  $684 \pm 14$  in 1918, and its highest value was  $921 \pm 33$  in 1917.

Crested wheatgrass had a water requirement of  $880 \pm 10$  in 1913 and  $1,024 \pm 34$  in 1914. Its water requirement was higher than that of brome grass ( $852 \pm 26$ ) in 1913, the only year in which both were grown. The low water requirement ( $422 \pm 13$ ) for the early-summer crop in 1914 and the high value ( $1,705 \pm 195$ ) for the mid-to-late summer crop are due to its habit of growth. This grass normally produces only one crop very early in the season and recovers slowly, making sparse growth during the summer and autumn. It is like yellow-flowered alfalfa in this respect.

Western wheatgrass produced a small crop, 28.6 gm. of dry matter per pot, with a high water requirement,  $1,247 \pm 31$ , in 1913. One pot overwintered gave a value of 1,420 in 1914.

## SUGAR BEET

One variety of sugar beet (*Beta vulgaris*), Kleinwanzleben, was grown in 1918. The plants made normal growth, the weight of fresh roots being 653 gm. (23.1 ounces) per pot. The water requirement based on total dry matter including tops was  $304 \pm 6$ . (Table 18.) The value based on the weight of dry roots was  $451 \pm 18$ .

TABLE 18.—Water requirement of Kleinwanzleben sugar beet at Newell, S. Dak., in 1918

Period of growth	Pot No.	Dry matter	Roots (dry)		Water	Water requirement based on—	
						Roots (dry)	Dry matter
June 15 to Oct. 9 (116 days).....		<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>	<i>Kgm</i>		
	49	169.6	109.3	65	50.6	463	298
	50	150.3	100.5	67	47.6	474	317
	51	181.0	129.0	71	51.4	399	284
	52	165.3	101.0	61	56.5	560	342
	53	192.6	139.2	72	57.7	414	300
	54	203.1	146.2	72	57.9	396	285
Mean.....		177.0	120.9			$451 \pm 18$	$304 \pm 6$

## EXPERIMENTS AT MANDAN, N. DAK.

The experiments on the water requirement of crop plants were continued at the United States Northern Great Plains Field Station, Mandan, N. Dak., from 1919 to 1922. This station is located 2 miles south of Mandan, Morton County, near the confluence of the Heart River with the Missouri, approximately in latitude  $46^{\circ} 50' N.$  and longitude  $100^{\circ} 55' W.$  The altitude at the field station is about 1,750 feet. The soil of the region is a brown to black loam derived from glacial till. The native vegetation is a mixture of the tall-grass and short-grass types, the latter generally predominating. According to Sarvis (10) the dominant species in the native vegetation are grama grass (*Bouteloua gracilis*), needle-and-thread grass (*Stipa comata*), and two small early sedges, *Carex filifolia* and *C. heliophila*. Many other grasses, including *Koeleria cristata*, *Agropyron smithii*, *A. tenerum*, *Andropogon scoparius*, *Stipa viridula*, *S. spartea*, and a variety of dicotyledons make a rich and varied flora.

The climate at Mandan is very similar to that at Newell. Although Mandan is approximately 2 degrees north of Newell, its altitude is 1,100 feet lower. The difference in soil between the two areas is most significant, the lighter loam soils at Mandan apparently being more favorable for native vegetation and for agricultural crops than the heavy clay soils at Newell.

The average annual precipitation at Mandan is approximately 16 inches (see Table 1) and the seasonal rainfall (April to August, inclusive) 11.4 inches. Table 19 shows a summary of the weather conditions for the six months April to September, inclusive, during the four years of the experiments, 1919 to 1922. The evaporation, by 5-day periods during the same months, is shown in Table 20. The relation of evaporation to the water requirement is discussed later in this paper.

TABLE 19.—Summary of monthly (April to September, inclusive) climatic conditions, 1919–1922, at Mandan, N. Dak.

Year and month	Air temperature					Average wind velocity per hour	Precipi- tation	Evapo- ration
	Average daily—			Absolute—				
	Mean	Maxi- mum	Mini- mum	Maxi- mum	Mini- mum			
1919:	° F.	° F.	° F.	° F.	° F.	Miles	Inches	Inches
April.....	43	54	32	75	24	7.4	1.72	3.74
May.....	56	69	43	93	20	7.0	3.95	6.02
June.....	69	83	56	102	32	6.4	1.12	7.26
July.....	74	89	59	100	47	5.4	.85	9.17
August.....	71	87	55	100	46	5.6	1.22	8.06
September.....	61	74	47	94	33	6.1	.49	5.35
Average or total.....	62	76	49			6.3	9.35	39.60
1920:								
April.....	35	47	24	60	0	6.7	0.38	3.16
May.....	56	69	43	88	34	7.9	1.72	5.54
June.....	66	79	52	93	36	5.7	1.85	6.30
July.....	71	85	58	103	50	4.6	2.68	7.16
August.....	71	86	55	100	39	5.3	1.81	8.45
September.....	60	73	46	93	25	4.4	1.29	4.65
Average or total.....	60	73	46			5.8	9.93	35.26
1921:								
April.....	43	56	30	83	14	6.3	2.59	3.64
May.....	55	67	43	89	27	6.3	3.05	5.15
June.....	71	84	58	108	41	5.6	.82	7.32
July.....	74	89	59	110	52	4.7	3.38	8.90
August.....	71	86	56	102	43	5.4	.25	8.52
September.....	59	73	46	95	31	7.1	1.58	5.73
Average or total.....	62	76	49			5.9	11.67	39.26
1922:								
April.....	45	56	33	89	15	6.4	0.66	3.38
May.....	58	70	46	81	36	7.2	2.05	5.34
June.....	66	78	54	96	45	4.3	3.43	5.64
July.....	68	82	55	96	38	3.8	3.17	6.74
August.....	73	87	58	101	49	4.7	.32	7.94
September.....	61	75	48	98	33	4.5	2.31	4.83
Average or total.....	62	75	49			5.2	11.94	33.87

## WATER REQUIREMENT OF CROP PLANTS

## WHEAT

Kubanka wheat (*Triticum durum*) was grown during the four seasons 1919 to 1922. The detailed results are shown in Table 21.

The water requirement of wheat was low in two years— $340 \pm 4$  in 1920 and  $372 \pm 8$  in 1922—and high in 1919 ( $520 \pm 11$ ) and in 1921 ( $351 \pm 8$ ). The high values in 1919 and 1921 are correlated with high evaporation during the months of June and July in those years as compared with the same months in 1920 and 1922. (Table 20.) The close correlation between evaporation and water requirement of Kubanka wheat is shown graphically in Figure 10. A view of some of the plants grown in 1922 is shown in Figure 6.



FIGURE 6.—Baltic alfalfa (left foreground), varieties of wheat (background), and weeds (right foreground), in the water-requirement experiments at Mandan, N. Dak., July 26, 1922

TABLE 20.—Evaporation by 5-day periods from April to September, inclusive, 1919-1922, at Mandan, N. Dak.

Period (dates inclusive)	Seasonal evaporation for—				Period (dates inclusive)	Seasonal evaporation for—			
	1919	1920	1921	1922		1919	1920	1921	1922
April:	Inches	Inches	Inches	Inches	July:	Inches	Inches	Inches	Inches
1-5.....	0.409	0.351	0.772	0.309	1-5.....	1.431	1.031	1.501	1.235
6-10.....	.418	.502	.146	.363	6-10.....	1.821	1.017	1.702	1.043
11-15.....	.378	.594	.771	.437	11-15.....	1.398	1.013	1.308	1.125
16-20.....	.899	.624	.880	.455	16-20.....	1.783	1.069	1.234	1.114
21-25.....	.852	.380	.397	.913	21-25.....	1.301	1.452	1.807	1.076
26-30.....	.783	.804	.672	.902	26-31.....	1.434	1.575	1.347	1.147
Total.....	3.739	3.155	3.638	3.379	Total.....	9.168	7.157	8.899	6.740
May:					August:				
1-5.....	0.531	0.181	1.051	0.899	1-5.....	1.257	1.167	1.420	1.200
6-10.....	.568	.833	.722	.621	6-10.....	1.293	1.421	1.305	1.096
11-15.....	.978	1.181	.762	.662	11-15.....	1.370	1.116	1.223	1.683
16-20.....	.792	1.027	.837	.958	16-20.....	1.610	1.867	1.368	1.455
21-25.....	1.174	1.144	1.036	1.104	21-25.....	1.087	1.505	1.409	1.396
26-31.....	1.979	1.177	.739	1.093	26-31.....	1.440	1.376	1.799	1.106
Total.....	6.022	5.543	5.147	5.337	Total.....	8.057	8.452	8.524	7.935
June:					September:				
1-5.....	0.993	0.830	0.873	1.344	1-5.....	0.870	0.438	1.551	0.989
6-10.....	.925	1.168	.786	.634	6-10.....	.909	.695	.916	1.171
11-15.....	1.136	1.135	1.130	.745	11-15.....	1.095	1.238	.250	.399
16-20.....	1.229	1.335	1.411	.875	16-20.....	1.106	.846	.878	.592
21-25.....	1.252	1.034	1.461	1.056	21-25.....	.944	.722	1.019	.777
26-30.....	1.723	.795	1.663	.984	26-30.....	.423	.708	1.116	.901
Total.....	7.258	6.297	7.324	5.638	Total.....	5.347	4.647	5.730	4.826
					Total for season.....	39.591	35.251	39.262	33.855

TABLE 21.—Water requirement of Kubanka wheat (C. I. 1440), 1919–1922, at Mandan, N. Dak.

Year and period of growth	Pot No.	Dry matter	Grain		Water	Water requirement based on—	
						Grain	Dry matter
		<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>	<i>Kgm.</i>		
1919 May 16 to Aug. 6 (81 days) -----	1	111.2	33.0	30	58.5	1,773	526
	2	91.7	17.5	19	44.5	2,543	485
	3	104.4	32.2	31	50.8	1,578	487
	4	127.3	24.1	19	64.2	2,664	504
	5	116.8	20.3	17	63.1	3,108	540
	6	130.1	25.4	20	75.1	2,957	577
Mean -----		113.6	25.4			2,437±192	520±11
1920. May 19 to Aug. 1b (78 days) -----	1	153.0	50.0	33	48.3	966	316
	2	145.2	40.2	28	48.6	1,208	335
	3	142.0	42.0	30	50.3	1,198	354
	4	153.4	52.7	34	51.8	983	338
	5	149.0	54.0	36	51.4	952	345
	6	145.7	51.5	35	51.5	1,000	353
Mean -----		148.1	48.4			1,051±38	340±4
1921 <sup>a</sup> May 18 to July 31 (75 days) -----	1	94.0	( <sup>a</sup> )		52.8		562
	2	133.0			70.8		528
	3	113.5			62.3		549
	4	110.4			56.7		514
	5	115.0			57.6		501
Mean -----		113.2					531±8
1922: May 16 to Aug. 14 (90 days) -----	1	258.7	94.0	36	104.3	1,110	403
	2	202.1	76.7	38	73.4	957	363
	3	194.0	72.1	37	75.0	1,040	387
	4	218.4	84.3	39	74.0	878	339
	5	199.7	75.4	38	73.9	980	370
Mean -----		214.6	80.5			991±18	372±8

<sup>a</sup> Unfortunately some loss of grain, due to damage by birds, occurred a day or two before harvesting the plants in 1921. The yield of dry matter, therefore, should have been somewhat greater than that actually obtained, and the water requirement somewhat lower than indicated. Probably the true value was between 500 and 520.

In 1922 four distinct types of wheat were grown in order to determine their relative economy in the use of water. The varieties were Kubanka, Marquis, Kota, and Hard Federation. They were grown in pots of soil which had been planted to flax the previous year. The results are shown in Table 22. The yield of grain and of total dry matter was satisfactory and much the same in the four varieties. Kubanka gave the lowest value,  $311 \pm 4$ , based on total dry matter, followed by Marquis,  $355 \pm 7$ ; Kota,  $393 \pm 6$ ; and Hard Federation,  $405 \pm 4$ . The water requirement of the four varieties, based on grain, showed a similar variation, ranging from  $847 \pm 11$  for Kubanka to  $1,124 \pm 20$  for Hard Federation. The yields in field plots varied in the same order as the water requirement, except for the Marquis variety, which was fourth in yield. The yields of grain in replicated field plots in bushels per acre were as follows: Kubanka, 19.6; Kota, 18.6; Hard Federation, 18.1; and Marquis, 16.7.

TABLE 22.—Water requirement in 1922 of four varieties of wheat grown in pots of soil which had been planted to flax the previous year (1921), at Mandan, N. Dak.

Plant and period of growth	Pot No.	Dry matter	Grain		Water	Water requirement based on—	
						Grain	Dry matter
TRITICUM DURUM							
Kubanka, C. I. 1440, May 27 to Aug. 8 (86 days)	6	148.5	53.3	36	45.8	859	308
	7	142.3	55.7	39	42.6	765	299
	8	208.7	71.6	34	68.3	954	327
	9	161.3	62.3	39	48.7	781	302
	10	181.3	63.7	36	57.7	878	318
Mean		168.4	61.7			847±11	311±4
T. VULGARE							
Hard Federation C. I. 4733, May 27 to Aug. 16 (81 days)	11	153.9	56.7	37	63.5	1,120	413
	12	181.2	66.6	37	74.0	1,111	408
	13	156.4	59.8	38	62.8	1,050	402
	14	142.5	50.0	35	54.7	1,094	384
	15	150.6	50.8	34	63.1	1,242	419
Mean		156.9	56.8			1,124±20	405±4
Kota, C. I. 5878, May 27 to Aug. 18 (83 days)	16	180.6	68.7	38	70.0	1,019	388
	17	198.8	75.6	38	75.4	997	379
	18	157.4	58.6	37	63.7	1,087	405
	19	159.2	62.1	39	69.2	953	372
	20	186.8	68.9	37	78.2	1,135	419
Mean		176.6	66.8			1,038±25	393±6
Marquis, C. I. 3641, May 27 to Aug. 16 (81 days)	21	156.5	61.1	39	54.9	899	351
	22	162.9	62.2	38	59.7	960	366
	23	163.0	58.5	37	63.3	1,082	388
	24	154.5	60.0	39	51.7	862	335
	25	164.5	65.4	40	55.0	841	334
Mean		160.3	61.4			929±31	355±7

## MILLET

Kursk millet, A. D. I. 3 (*Chaetochloa italica*), was grown in 1919 and 1920. (Table 23.) Its water requirement based on dry matter was  $303 \pm 2$  in 1919 and  $186 \pm 1$  in 1920. The value based on grain in 1919 was  $1,337 \pm 40$ . In 1920 the millet was harvested before the seed was fully mature, and the water requirement based on grain was not determined.

TABLE 23.—Water requirement of Kursk millet (A. D. I. 3) in 1919 and 1920, at Mandan, N. Dak.

Period of growth	Pot No.	Dry matter	Grain		Water	Water requirement based on—	
						Grain	Dry matter
1919: June 15 to Aug. 21 (67 days)-----	31	258.2	62.0	24	78.0	1,259	302
	32	252.2	53.1	21	77.5	1,460	307
	33	245.7	54.2	22	74.0	1,365	301
	34	243.8	50.0	21	75.3	1,506	309
	35	254.2	63.1	25	75.9	1,203	299
	36	281.2	66.8	24	82.0	1,228	292
Mean-----		255.9	58.2			1,337±40	303±2
1920: June 15 to July 31 (64 days)-----	7	198.0			36.9		186
	8	229.8			41.3		180
	9	213.5			39.7		186
	10	201.0			38.1		189
	11	216.8			39.1		180
	12	194.5			37.8		194
Mean-----		208.9					186±1

## SORGO AND SUDAN GRASS

Dakota Amber sorgo (*Sorghum vulgare*) and Sudan grass (*S. vulgare* var. *sudanense*) were grown in 1919 and 1920. In 1920 an  $F_3$  hybrid of those varieties also was grown. The results are shown in Table 24.

As in the results with wheat and millet, a higher water requirement was recorded in 1919 than in 1920. The average water requirement of Sudan grass ( $311 \pm 4$ ) for the 2-year period was 24 per cent higher than that of Dakota Amber sorgo ( $251 \pm 2$ ). The hybrid ( $224 \pm 1$ ) gave approximately the same value as sorgo in 1920.

TABLE 24.—Water requirement of Dakota Amber sorgo and Sudan grass in 1919 and 1920, and of an  $F_3$  hybrid of Dakota Amber sorgo  $\times$  Sudan grass in 1920, at Mandan, N. Dak.

Plant and period of growth	Pot No.	Dry matter	Grain		Water	Water requirement based on—	
						Grain	Dry matter
DAKOTA AMBER SORGO							
1919: June 15 to Aug. 21 (67 days)-----	25	285.8	107.2	38	77.2	720	270
	26	263.0	106.0	40	72.4	683	275
	27	250.0	98.1	39	69.1	704	276
	28	279.2	101.2	36	75.4	745	270
	29	229.4	65.8	29	63.3	962	276
	30	260.1	99.0	38	70.1	708	270
	Mean-----		261.3	96.2			712±5
1920: June 15 to Aug. 18 (64 days)-----	25	184.7			41.6		225
	26	173.7			40.8		235
	27	186.0			41.1		221
	28	178.0			40.9		230
	29	173.8			41.2		237
	30	168.8			38.2		226
	Mean-----		177.5				
SUDAN GRASS							
1919: June 15 to Aug. 21 (67 days)-----	37	204.2			71.2		349
	38	232.4			77.4		333
	39	220.1			75.1		341
	40	231.2			78.1		338
	41	217.7			78.5		361
	42	226.2			80.9		338
	Mean-----		222.0				
1920: June 25 to Aug. 18 (64 days)-----	13	202.7			55.8		275
	14	219.8			56.9		259
	15	193.1			55.7		288
	16	203.6			57.1		280
	17	211.3			56.7		268
	18	207.8			56.7		273
	Mean-----		206.4				
DAKOTA AMBER SORGO × SUDAN GRASS, F <sub>3</sub>							
1920: June 15 to Aug. 18 (64 days)-----	37	152.5			34.1		224
	38	157.9			34.9		221
	39	148.0			33.4		226
	40	161.7			36.2		224
	41	156.3			34.6		221
	42	156.7			35.2		225
Mean-----		155.5					224±1

<sup>a</sup> Not included in the mean.



## ALFALFA

In the experiments at Mandan less attention was given than at Newell to the comparative water requirement of different varieties of alfalfa. Instead of determining the water requirement of many varieties, an effort was made to determine the effect of time of cutting, that is, the period of growth, on the water requirement of alfalfa. This subject is discussed under the heading "Effect of Cutting Alfalfa at Different Stages of Growth" on page 2.

The water requirement of Grimm, A. D. I. E-23, as grown to maturity for seed in 1921, and that of Baltic, A. D. I. H-4-58, cut twice in 1922, are shown in Table 25.

TABLE 25.—Water requirement of Grimm alfalfa (A. D. I. E-23), grown to maturity for seed in 1921, and of Baltic alfalfa (A. D. I. H-4-58), cut in early-bloom stage, in 1922, at Mandan, N. Dak.

Plant and period of growth	Pot. No.	Dry matter	Water	Water requirement based on dry matter
GRIMM, A. D. I. E-23				
1921. June 9 to Sept. 2 (85 days).....	26	Grams 94.0	Kgm. 94.2	1,002
	27	90.0	95.0	1,055
	28	102.0	96.6	947
	29	100.5	82.2	818
	30	105.4	90.3	857
Mean.....		98.4		930±33
BALTIC, A. D. I. H-4-58				
1922: Midsummer June 1 to July 26 (55 days).....	26	50.1	36.9	737
	27	50.4	39.8	790
	28	68.4	52.8	772
	29	80.2	61.2	763
	30	47.7	42.9	809
Mean.....		59.4		792±18
Late summer, July 26 to Sept. 1 (37 days).....	26	55.3	41.1	743
	27	64.7	41.4	610
	28	80.5	58.6	728
	29	95.0	64.4	678
	30	65.0	47.0	723
Mean.....		72.1		702±15
Combined crop, June 1 to Sept. 1 (92 days).....	26	105.4	78.0	740
	27	115.1	81.2	705
	28	148.9	111.4	748
	29	175.2	125.6	717
	30	112.7	89.9	798
Mean.....		131.5		742±11

## FLAX

Four varieties of flax (*Linum usitatissimum*) were grown in 1921 and six varieties in 1922 in order to determine the water requirement of this crop as grown for seed production (linseed). The data are shown in Table 26.

TABLE 26.—*Water requirement of varieties of seed flax in 1921 and 1922, at Mandan, N. Dak.*

Plant and period of growth	Pot No.	Dry matter	Seed		Water	Water requirement based on—	
						Seed	Dry matter
DAMONT, C. I. 3							
1921: June 9 to Sept. 2 (85 days)-----	6	114.8	34.5	30	89.4	2,590	778
	7	107.2	28.3	26	81.3	2,870	758
	8	87.5	28.1	32	78.1	2,780	892
	9	112.7	36.0	32	97.2	2,700	862
	10	111.8	36.8	33	90.4	2,457	808
Mean-----		106.8	32.7			2,679±53	820±19
1922: June 13 to Oct. 5 (114 days)-----	51	85.6	30.4	36	75.6	2,487	883
	52	78.8	27.3	35	71.1	2,604	902
	53	58.0	19.3	33	46.4	2,404	800
	54	56.1	20.2	36	52.8	2,614	941
	55	78.0	26.7	34	67.1	2,513	860
Mean-----		71.3	24.8			2,524±29	877±11
RESERVE C. I. 19							
1921: June 9 to Sept. 2 (85 days)-----	21	104.3	34.0	33	86.3	2,540	826
	22	101.1	33.8	33	89.2	2,639	882
	23	113.5	34.1	30	90.7	2,660	800
	24	111.4	34.2	31	90.4	2,643	812
	25	117.5	35.1	30	90.5	2,580	770
Mean-----		109.6	34.2			2,612±18	818±12
1922: June 13 to Oct. 5 (114 days)-----	56	86.3	28.1	33	78.0	2,776	904
	57	75.1	25.1	33	65.3	2,602	870
	58	72.9	23.8	33	64.5	2,710	885
	59	85.7	28.8	34	69.6	2,417	812
	60	82.3	28.6	35	65.7	2,297	798
Mean-----		80.4	26.9			2,560±69	854±16
PRIMOST, C. I. 12							
1921: June 9 to Aug. 22 (74 days)-----	11	86.3	22.6	26	65.3	2,890	757
	12	91.1	25.0	27	69.1	2,765	759
	13	87.0	23.4	27	64.6	2,760	743
	14	90.0	23.5	26	67.5	2,870	750
	15	88.0	24.5	28	64.2	2,620	730
Mean-----		88.5	23.8			2,781±33	748±4
1922: June 13 to Oct. 5 (114 days)-----	76	76.5	25.8	34	57.7	2,236	754
	77	84.2	30.5	36	70.5	2,311	837
	78	80.2	27.5	34	64.4	2,342	803
	79	67.5	21.9	32	54.1	2,470	801
	80	81.5	28.7	35	65.9	2,296	808
Mean-----		78.0	26.9			2,331±26	801±8
NORTH DAKOTA RESISTANT NO. 114, C. I. 13							
1921: June 9 to Aug. 22 (74 days)-----	16	79.0	18.3	23	62.4	3,410	790
	17	82.6	19.3	23	60.7	3,145	735
	18	81.5	19.7	24	60.6	3,075	744
	19	76.7	18.2	24	58.3	3,203	760
	20	74.5	19.7	26	56.0	2,845	752
Mean-----		78.9	19.0			3,135±59	756±6
1922: June 13 to Oct. 5 (114 days)-----	71	77.7	24.5	32	51.4	2,098	662
	72	71.2	26.1	37	54.3	2,080	763
	73	69.7	27.1	39	55.5	2,048	796
	74	57.2	20.1	35	40.7	2,025	712
	75	58.2	20.5	35	43.1	2,102	741
Mean-----		66.8	23.7			2,070±11	735±12

TABLE 26.—*Water requirement of varieties of seed flax in 1921 and 1922, at Mandan, N. Dak.*—Continued

Plant and period of growth	Pot No.	Dry matter	Seed		Water	Water requirement based on	
						Seed	Dry matter
WINONA, C. I. 179							
1922: June 13 to Oct. 5 (114 days)-----	66	61.5	25.0	39	44.6	1,784	691
	67	68.7	26.6	39	50.5	1,898	736
	68	61.3	20.5	33	45.5	2,219	742
	69	72.9	27.8	38	57.3	2,061	786
	70	69.0	23.7	34	50.7	2,139	735
Mean -----	--	67.3	24.7	--	-----	2,020±61	738±9
LETHBRIDGE GOLDEN, C. I. 23							
1922: June 13 to Oct. 5 (114 days)-----	61	67.5	26.0	39	62.8	2,415	930
	62	73.1	26.0	36	64.8	2,492	886
	63	65.3	23.3	36	58.5	2,511	896
	64	72.0	27.5	38	64.8	2,356	900
	65	74.1	27.0	36	66.7	2,470	900
Mean -----		70.4	26.0	--	-----	2,449±18	902±5

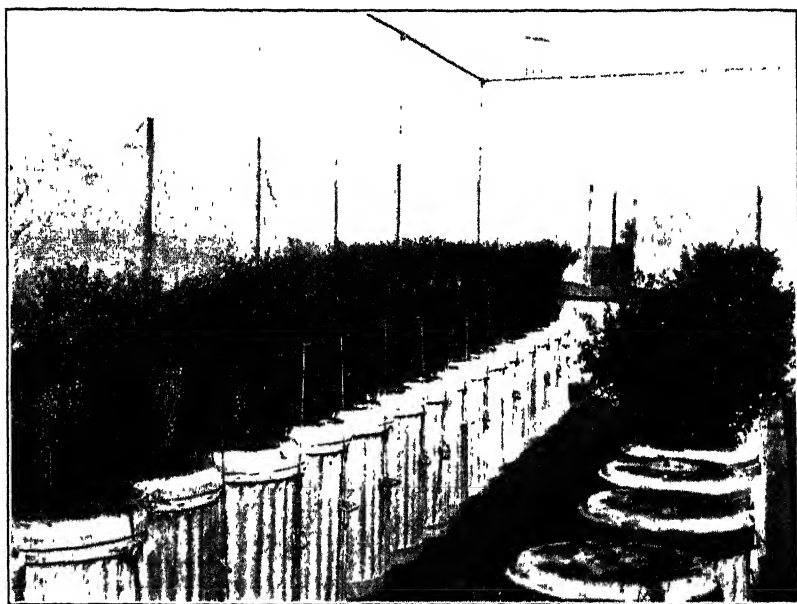


FIGURE 7.—Flax varieties (at left) and Grimm alfalfa grown in the water-requirement experiments at Mandan, N. Dak. Photographed August 22, 1921

The two varieties Damont and Reserve are of similar type—the so-called Russian or European blue-flowered seed flaxes, which formerly were grown extensively on new lands in the Dakotas and Montana. The three varieties N. D. R.<sup>7</sup> 114, Primost, and Winona are small seeded and more or less wilt resistant. Lethbridge Golden is not grown commercially. It has large chamois-yellow seeds and pale-pink flowers. (Fig. 7.)

<sup>7</sup> North Dakota Resistant.

Based on dry matter, the varieties Primost and N. D. R. 114 had a somewhat lower water requirement than Damont and Reserve, the values ranging from 746 for N. D. R. 114 to 848 for Damont. Based on seed production, their water requirements were very similar, ranging from 2,556 for Primost to 2,602 for N. D. R. 114.

The yield of flaxseed in these pot experiments was satisfactory and apparently quite normal, the calculated yields per acre ranging from about 12 to 22 bushels.



FIGURE 8.—Some common weeds (witch grass, redroot pigweed, lamb's-quarters, and Russian thistle) grown in the water-requirement experiments at Mandan, N. Dak.

#### WATER REQUIREMENT OF WEEDS

In 1919 and in 1922 a few common weeds were grown in the water-requirement experiments. The data are shown in Table 27.

Although the experiments were few, the results indicate that Russian thistle (*Salsola tenuifolia*), redroot pigweed (*Amaranthus retroflexus*), and witch grass (*Panicum capillare*) are highly efficient in the use of water. A photograph of redroot pigweed and lamb's-quarters (*Chenopodium album*) taken on the day the plants were cut, July 26, 1922, is shown in Figure 8.

TABLE 27.—*Water requirement of five common weeds in 1919 and 1922 at Mandan, N. Dak.*

Plant and period of growth	Pot No.	Dry matter	Water	Water requirement based on dry matter
<b>RUSSIAN THISTLE (<i>SALSOLA TENUIFOLIA</i>)</b>				
1919: June 15 to July 16 (31 days).....	43	<i>Grams</i> 118 0	<i>Kgm.</i> 25 9	218
	44	102.6	26.0	253
	45	112 1	26 7	238
Mean.....		110 9		237±7
July 31 to Sept. 11 (42 days).....	45	44.3	11.8	266
1922: June 12 to July 26 (44 days).....	85	227.2	45.4	200
	86	228 5	47.6	208
Mean.....		227 9		204±3
<b>REDROOT PIGWEED (<i>AMARANTHUS RETROFLEXUS</i>)</b>				
1919: June 15 to July 16 (31 days).....	46	78.5	18.8	240
	47	59.4	16.9	284
	48	53.2	15.5	291
Mean.....		63 7		272±12
1922: June 12 to July 26 (44 days).....	83	202.1	48.9	242
<b>WITCH GRASS (<i>PANICUM CAPILLARE</i>)</b>				
1919: July 31 to Sept. 15 (46 days).....	43	11.6	3 5	302
	44	25.0	6.6	204
Mean.....		18.3		283±16
1922: June 12 to July 26 (44 days).....	81	186 6	41 8	224
	82	137 7	30.0	218
Mean.....		162.2		221±3
<b>PURSLANE (<i>PORTULACA OLERACEA</i>)</b>				
1919: July 28 to Sept. 11 (45 days).....	46	20.7	6 7	324
	47	24.3	7.2	296
	48	28.5	9.4	330
Mean.....		24.5		317±8
<b>LAMB'S-QUARTERS (<i>CHENOPodium ALBUM</i>)</b>				
1922: June 6 to July 26 (44 days).....	84	214.1	82.0	383

## EFFECT OF CUTTING ALFALFA AT DIFFERENT STAGES OF GROWTH

In experiments at Akron, Colo., in 1912, Briggs and Shantz (2) found that when alfalfa was clipped at weekly intervals the water requirement was somewhat higher ( $975 \pm 23$ ) than when it was cut in the usual manner, that is, in the early-blossom stage for hay ( $853 \pm 13$ ). The total yield of dry matter was much less in the series frequently clipped than in the series cut for hay, the yields being 27 and 128 gm. per pot, respectively. The growth period covered 42 days, from July 26 to September 6, the one series being clipped six times, the other only once.

In 1919 the writer carried on a similar but more extensive experiment at Mandan, N. Dak. Three sets of pots of Grimm alfalfa, A. D. I. E-23, were grown in order to determine the water requirement of alfalfa under three conditions of cutting: (1) When the plants are allowed to mature seed; (2) when the plants are cut in the early blossom stage, as for hay; and (3) when the plants are clipped at intervals of two weeks, simulating pasture conditions. A duplicate experiment was conducted with yellow-flowered alfalfa, *Medicago falcata*.

The water requirement of Grimm alfalfa, A. D. I. E-23, when harvested in the three stages of growth described above, is shown in Table 28.

TABLE 28.—Water requirement of *Grimm alfalfa* (A. D. I. E-28), in 1919, at Mandan, N. Dak.: (A) When matured for seed; (B) when cut in early bloom for hay; and (C) when cut at intervals of two weeks, to imitate pasturing

Series A —Matured for seed					Series B.—Harvested in early-bloom stage					Series C.—Cropped at intervals				
Period of growth	Pot No.	Dry matter	Water absorbed	Water requirement	Period of growth	Pot No.	Dry matter	Water absorbed	Water requirement	Period of growth	Pot No.	Dry matter	Water absorbed	Water requirement
		Grams	Kgm.				Grams	Kgm.				Grams	Kgm.	
Early midsummer and June 15 to Aug. 22 (68 days).	49	104.5	94.0	900	Early summer, June 15 to July 19 (34 days).	55	56	30.9	757	Early summer, June 15 to July 19 (34 days).	61	21.6	16.7	773
	50	103.3	94.5	915		56	33.2	24.3	711		62	35.3	24.9	705
	51	102.4	88.2	861		57	38.4	24.4	733		63	32.2	23.0	780
	52	84.6	87.3	1,032		58	38.4	24.4	733		64	26.7	22.0	771
	53	93.9	93.7	1,008		59	32.6	26.9	825		65	36.9	27.8	753
	54	89.3	79.2	887		60	40.2	28.0	697		66	35.5	24.9	702
Mean.....					Mean.....				747±12	Mean.....				747±11
Late summer, Aug. 22 to Oct. 7 (46 days).	49	59.6	44.3	743	Midsummer, July 19 to Aug. 22 (34 days).	55	53.7	39.7	739	Midsummer, July 19 to Aug. 22 cropped Aug 2, 16, and 22.	61	36.0	19.8	550
	50	60.8	43.3	712		56	60.5	43.1	712		62	53.3	33.4	627
	51	57.8	44.5	770		57	48.4	32.9	680		63	51.4	32.8	638
	52	50.6	41.5	820		58	62.5	47.0	752		64	56.9	36.6	643
	53	57.0	41.6	730		59	50.4	36.9	732		65	45.0	30.1	669
	54	45.7	32.6	713		60	61.0	42.9	703		66	45.8	31.6	690
Mean.....				932±21	Mean.....				720±8	Mean.....				636±11
Combined crop, June 15 to Oct. 7 (114 days).	49	164.1	138.3	843	Late summer, Aug 22 to Oct. 7 (46 days).	55	50.7	35.9	708	Late summer, Aug 22 to Oct. 7 cropped Sept. 15 and Oct. 7.	61	33.4	17.6	527
	50	164.1	137.8	840		56	55.7	36.4	654		62	42.9	23.0	536
	51	160.2	132.7	828		57	47.2	30.6	648		63	39.6	21.8	551
	52	135.2	128.8	952		58	61.5	38.8	631		64	33.8	23.5	695
	53	150.9	135.3	897		59	48.6	33.8	695		65	40.8	22.1	542
	54	135.0	111.8	828		60	49.2	32.8	667		66	42.3	23.8	563
Mean.....				748±12	Mean.....				667±9	Mean.....				549±10
Combined crop, June 15 to Oct. 7 (114 days).	49	164.1	138.3	843	Combined crop, June 15 to Oct. 7 (114 days).	55	134.4	98.3	731	Combined crop, June 15 to Oct. 7 (114 days).	61	91.0	54.1	595
	50	164.1	137.8	840		56	130.8	103.8	690		62	131.5	81.3	615
	51	160.2	132.7	828		57	128.8	97.2	682		63	128.4	80.7	680
	52	135.2	128.8	952		58	132.4	107.2	703		64	128.7	80.0	682
	53	150.9	135.3	897		59	131.0	97.7	742		65	129.7	80.3	652
	54	135.0	111.8	828		60	130.4	105.7	680		66	128.6	80.3	650
Mean.....				865±15	Mean.....				706±8	Mean.....				642±9

The water requirement of the seed-producing crop (series A, Table 28) grown during the period June 15 to August 22 was  $932 \pm 21$  as compared with  $730 \pm 10$  for the two crops cut in the early bloom stage (series B) and covering exactly the same period of growth. Nearly the same difference was obtained with the yellow-flowered alfalfa (*Medicago falcata*) shown in Table 29. The water requirement of the seed crop of *M. falcata* (series A), covering the period June 20 to August 22, was  $884 \pm 19$ , as compared with  $731 \pm 10$ , the combined value of the two crops cut in the early-bloom stage and covering the same period of growth.

The seed crop of Grimm alfalfa required 28 per cent more water to produce a unit quantity of dry matter than did the combined crops grown during the same period and cut in the proper stage for hay. The seed crop of *Medicago falcata* required 21 per cent more water than the combined hay crop to produce a unit quantity of dry matter.

In 1921, Grimm alfalfa, A. D. I. E-23, grown to maturity for seed, had a water requirement of  $936 \pm 33$ . (See Table 25.)

In an earlier experiment at Newell, S. Dak., in 1918, Grimm alfalfa, A. D. I. E-23, grown for seed, had a water requirement of  $1,399 \pm 14$ , as compared with the combined value  $955 \pm 10$  of two crops cut in early bloom. (See Table 13.) The growth period covered 99 days—June 25 to October 2—in both cases. In this experiment the seed crop required 46 per cent more water to produce a unit quantity of dry matter than did the combined crops cut in the proper stage of growth for hay.

In series B and series C (Table 28) the first or early-summer crop was grown to the early-bloom stage, and both sets had exactly the same water requirement,  $747 \pm 12$ . During the second period, midsummer, series B was cut only once, on August 22 at the end of the 34-day period, while series C was cropped three times, on August 2, 16, and 22. The water requirement of series B was  $720 \pm 8$  as compared with  $636 \pm 11$  for series C.

In the late summer, August 22 to October 7, series B, cut once, had a water requirement of  $667 \pm 9$ , as compared with  $569 \pm 10$  in series C, which was cropped twice, on September 15 and October 7.

In yield of dry matter, the two series produced approximately the same quantity during the first or early-summer period—33.1 gm. per pot in series B and 31.9 gm. in series C. This indicates that the two series were of equal vigor at the beginning of the experiment. In the midsummer period, series B yielded 56.1 gm. per pot, as compared with 48.1 gm. in series C. In the late-summer period, series B yielded 52.2 gm. per pot, as compared with 38.8 gm. in series C. The yield of series C, therefore, was 86 per cent of that of series B during the midsummer period and 74 per cent of series B during the late-summer period. The reduced leaf area of the plants, brought about through frequent cutting, reduced the total quantity of water transpired and also the quantity of dry matter produced by the plant.

The experiment with the yellow-flowered alfalfa (Table 29) was not so satisfactory, because of the slow seedling growth of this species and its slow recovery after cutting. The water requirement of series B—cut in early-bloom stage—was approximately the same as that of series C, which was cropped at more frequent intervals. The values for the midsummer period were  $717 \pm 14$  for series B and  $727 \pm 13$  for series C. In the late-summer period the values were  $734 \pm 20$  and  $753 \pm 18$ , respectively. The differences in either period are not significant.

TABLE 29.—Water requirement of yellow-flowered alfalfa in 1919, at Mandan N. Dak.: (A) When matured for seed (B) when cut in early bloom for hay; and (C) when cut at intervals of two weeks, to imitate pasturing

Series A.—Matured for seed					Series B.—Harvested in early-bloom stage					Series C.—Cropped at intervals of two weeks				
Period of growth	Pot No.	Dry matter	Water absorbed	Water requirement	Period of growth	Pot No.	Dry matter	Water absorbed	Water requirement	Period of growth	Pot No.	Dry matter	Water absorbed	Water requirement
		Grams	Kgm.				Grams	Kgm.				Grams	Kgm.	
Early and midsummer, June 20 to Aug. 22 (63 days).	79	53.9	50.4	753	Early summer, June 20 to July 19 (29 days)	73	15.7	11.9	758	Early summer, June 20 to July 19 (29 days).	67	8.5	7.7	906
	80	64.6	54.5	844		74	35.8	23.9	725		68	6.3	6.8	1,079
	81	51.4	44.7	870		75	13.2	10.7	811		69	8.5	8.1	953
	82	58.0	51.0	879		76	14.4	11.1	771		70	7.6	7.2	947
	83	76.7	61.0	795		77	37.4	27.0	722		71	8.3	6.6	795
Mean	84	59.0	56.9	964	Mean	78	33.8	26.5	784	Mean	72	11.5	8.8	765
		90.4		884±19			21.8		702±10			8.5		908±32
	79	22.3	19.8	888	Midsummer, July 19 to Aug. 22 (34 days).	73	40.2	33.1	824	Midsummer, cropped Aug. 2, 16, and 22 (34 days).	67	24.7	19.0	769
	80	18.5	18.1	978		74	35.8	30.3	683		68	23.9	18.6	778
	81	14.3	14.1	986		75	42.2	30.3	718		69	26.1	19.0	728
	82	16.2	17.5	1,080		76	53.1	36.4	685		70	23.7	18.7	662
	83	34.5	28.0	812		77	49.7	35.7	718		71	24.6	18.0	732
Late summer Aug. 22 to Oct. 7 (46 days).	84	24.4	23.5	963	Mean	78	43.4	29.9	689	Mean	72	28.5	19.7	691
		21.7		951±26			44.1		717±14			25.3		727±13
	79	22.3	19.8	888	Late summer, Aug. 22 to Oct. 7 (46 days).	73	24.5	20.0	816	Late summer, cropped Sept. 13 and Oct. 7 (46 days).	67	18.5	12.1	654
	80	18.5	18.1	978		74	24.8	20.3	744		68	19.8	7.9	806
	81	14.3	14.1	986		75	27.3	22.0	743		69	13.1	11.1	847
	82	16.2	17.5	1,080		76	36.2	22.0	686		70	10.7	8.5	794
	83	34.5	28.0	812		77	27.6	19.7	685		71	15.4	10.1	656
Mean	84	24.4	23.5	963	Mean	78	25.1	19.7	785	Mean	72	9.6	7.3	760
		21.7		951±26			27.6		734±20			12.8		753±18
	79	75.2	70.2	894	Combined crop, June 20 to Oct. 7 (109 days).	73	80.4	65.0	808	Combined crop, June 20 to Oct. 7 (109 days).	67	51.7	38.8	750
	80	83.1	72.6	874		74	76.6	54.2	708		68	40.0	33.3	832
	81	66.7	58.5	895		75	82.7	61.3	741		69	47.7	38.2	800
	82	74.2	68.5	893		76	103.7	69.5	670		70	42.0	31.4	748
	83	111.2	80.0	964		77	114.7	82.0	715		71	48.3	34.7	718
Mean	84	83.4	80.4	964	Mean	78	102.3	76.1	744	Mean	72	49.6	35.8	722
		82.1		808±16			93.4		731±13			46.6		702±14



The high water requirement of the early-summer crop in series C is due to the very low yield of dry matter. The plants, however, were well established and thrifty when the midsummer period began. A summary of the results presented in Tables 28 and 29 is shown in Table 30.

TABLE 30.—Summary of yield of dry matter and water requirement of Grimm and yellow-flowered alfalfas in 1919 at Mandan, N. Dak., (A) when matured for seed, (B) when cut in early bloom for hay, and (C) when cut at intervals to imitate pasturing

Plant and period of growth	Dry matter per pot			Water requirement		
	Series A	Series B	Series C	Series A	Series B	Series C
Grimm, A. T. I. E-23:	Grams	Grams	Grams			
June 15 to July 19.....		33 1	31.9		747±12	747±11
July 19 to Aug. 22.....	<sup>a</sup> 96 3	56.1	48.1	<sup>a</sup> 932±21	720±8	636±11
Aug. 22 to Oct. 7.....	55.3	52 2	38.8	748±12	667±9	569±10
Combined crop, June 15 to Oct. 7.....	151 6	141.3	118.7	865±15	706±8	612±9
Yellow-flowered alfalfa:						
June 20 to July 19.....		21 8	8 5		762±10	908±32
July 10 to Aug. 22.....	<sup>b</sup> 60 4	44 1	25.3	<sup>b</sup> 884±19	717±14	727±13
Aug. 22 to Oct. 7.....	21.7	27 6	12 9	951±20	734±20	753±18
Combined crop, June 20 to Oct. 7.....	82.1	93.4	46.6	898±16	731±13	762±14

<sup>a</sup> For the period June 15 to Aug. 22

<sup>b</sup> For the period June 20 to Aug. 22.

## EFFECT OF ENFORCED DROUGHT ON WATER REQUIREMENT AND YIELD OF OATS

It is a common observation that if drought occurs during the normally rapid growth period of a crop the yield of the crop is liable to be greatly reduced. It is of interest to know what effect enforced drought has on the water requirement. Does the crop use more or less water in proportion to the dry matter produced? Experiments bearing on this problem were conducted at Newell, S. Dak., in 1918 and at Mandan, N. Dak., in 1919.

Three sets of pots of Swedish Select oats (*Avena sativa*) were grown in a uniform manner except as to the method of watering. In series A (pots 7 to 12) the plants were watered normally—that is, water was added as often as necessary to keep the pots up to the original weight. In series B (pots 13 to 18) the plants were watered normally until the period of heading, when the soil was allowed to dry down until distinct wilting of the leaves occurred at midday on three or four successive days. Water was then added to bring the pots up to the original weight, which was maintained until the plants were ripe. In series C (pots 19 to 24) the plants were allowed to wilt at two periods, first when heading and again during the late milk stage. The data are shown in Table 31.

The most striking effect of the enforced drought was to cut down the yield of total dry matter and of grain. At Newell, 1918, the average yield of dry matter per pot was 120.6 gm. in series A, 80.2 gm. in series B, and 82.5 gm. in series C. At Mandan, 1919, the average yields were 125.8 gm., 102.4 gm., and 89.7 gm., respectively.

TABLE 31.—*Water requirement of Swedish Select oats in 1918 at Newell, S. Dak., and in 1919 at Mandan, N. Dak.: (A) When supplied with water throughout entire growth period, (B) when allowed to wilt severely during heading stage, and (C) when allowed to wilt during heading stage and again when grain was in late milk stage*

Location, year, experiment, and period of growth	Pot No.	Dry matter	Grain	Water	Water requirement based on—	
					Grain	Dry matter
NEWELL, S. DAK., 1918						
(A) Water supplied regularly—June 12 to Aug. 14 (63 days).	7	Grams 100 9	Grams 46.6	Kgm. 39.8	854	394
	8	113.4	55.4	46.1	832	406
	9	117.1	56.7	48.5	856	414
	10	153.3	66.1	66.5	1,007	434
	11	132.3	61.5	56.8	924	429
	12	106 4	38.0	51.7	1,360	486
	Mean.....		120.6	54.1	51.6	972±52
(B) Plants wilted in heading stage—June 12 to Aug. 14 (63 days).	13	82.0	31.5	36.3	1,152	443
	14	75.8	33.2	31.2	940	411
	15	84.6	30.8	36.0	1,168	426
	16	70.6	26.9	29.3	1,089	415
	17	92.3	45.2	38.7	856	419
	18	75 9	31 8	31.5	991	415
	Mean.....		80 2	33 2	33.8	1,033±39
(C) Plants wilted in heading and in late milk stages—June 12 to Aug. 10 (59 days)	19	73.1	35.2	24.0	632	329
	20	83.4	40.0	27.1	678	325
	21	81.6	39.1	28.1	719	345
	22	77.8	37.6	25.5	678	328
	23	86.3	41.7	27.3	655	317
	24	92.8	46.3	28.7	620	309
	Mean.....		82.5	40 0	26 8	672±8
MANDAN, N DAK., 1919						
(A) Water supplied regularly—June 2 to Aug 5 (64 days).	7	123.4	32.4	91.1	2,812	738
	8	123.7	37.6	82.7	2,199	669
	9	122.0	28.0	86.5	3,089	709
	10	123.3	31.1	90.9	2,923	737
	11	132.2	36.5	94.5	2,589	715
	12	130.4	37.5	89.4	2,384	686
	Mean.....		125.8	33.9	89.2	2,666±104
(B) Plants wilted in heading stage—June 2 to Aug. 2 (61 days)	13	104.8	19.2	73.8	3,844	704
	14	100.0	15.6	70.8	4,538	708
	15	101.1	20.2	70.2	3,475	694
	16	102.8	19.7	70.4	3,574	685
	17	100.3	18.2	71.3	3,918	711
	18	105.1	19.0	72.3	3,805	688
	Mean.....		102.4	18.7	71.5	3,859±93
(C) Plants wilted in heading and in late milk stages—June 2 to Aug. 2 (61 days).	19	86.4	20.2	57.3	2,837	663
	20	85.3	14.5	63.3	4,365	742
	21	81.5	19.1	60.8	3,183	746
	22	95.1	27.3	62.2	2,278	654
	23	94.8	30.0	61.7	2,057	651
	24	95.1	29.7	61.8	2,081	650
	Mean.....		89.7	23.5	61.2	2,800±185

The water requirement, based on dry matter, was practically the same in series A and B, but significantly lower in series C, at Newell in 1918. The values were  $427 \pm 9$ ,  $422 \pm 3$ , and  $326 \pm 3$ , respectively. At Mandan in 1919 the water requirement of the three series was the

same, within the range of experimental error. Because of a less favorable season, however, the values were higher than in 1918. The water requirement in series A was  $709 \pm 8$ ; series B,  $698 \pm 5$ ; and series C,  $684 \pm 15$ .

The water requirement based on grain ranged from  $672 \pm 8$  to  $1,033 \pm 39$  in 1918 and from  $2,666 \pm 104$  to  $3,859 \pm 93$  in 1919.

It will be seen (Table 31) that the yield of grain in series B, where the plants were allowed to wilt only at heading time, was less than in series C, where wilting occurred at two critical periods—the heading and milk stages. This apparent inconsistency can be explained by the observation that in series B the plants began a secondary growth of tillers, when water was supplied, after wilting in the heading stage, whereas, in series C the second period of soil drought had a tendency to limit the vegetative growth and to force maturity of the plants. This resulted in a higher yield of grain in series C than in series B.

The effect of enforced soil drought on the daily rate of transpiration is striking. The plants in series B, which transpired 0.56 inch of water per day during the period July 6 to 10, reduced transpiration to 0.22 inch during the period July 11 to 16. The mean daily transpiration of series C was reduced from 0.57 inch (July 6 to 10) to 0.28 inch during the first period of wilting (July 11 to 15) and to 0.17 inch during the second period (July 21 to 25).

In Figure 9 the mean daily transpiration by 5-day periods of each series in comparison with evaporation from a free water surface is shown. At the base of the graph is shown also the quantity of water added and the date of watering. In series A a high rate of transpiration was maintained for a period of about 20 days from July 5 to 25. In series B the rate of transpiration was only partly regained after the period of enforced drought, July 10 to 15, whereas in series C transpiration continued at a low rate after July 10 because of the two periods of enforced drought. The graph indicates quite clearly the reaction of crop plants to the soil-moisture supply.

The injurious effects of soil drought on the transpiration, growth, and yield of oats were evident from the facts that (1) when water was supplied after a period of wilting, transpiration continued at a low rate for three or four days; (2) the normal rate of transpiration was never regained, because of the reduced leaf area; (3) the vegetative growth of the plants was checked; and (4) the yield of dry matter and of grain was reduced. On the other hand, soil drought did not have a significant effect on the water requirement except in one case (series C, 1918) when the two periods of soil drought apparently lowered the water requirement as compared with series A and B. In this case there appears to have been some real adaptation of the plants to conditions of drought. This is in agreement with the results reported by Maksimov (8)—that a single period of wilting of the sunflower decreased the yield of dry matter to 40 per cent of the check, whereas subsequent wilting did not cause any further significant injury to the plants.

#### EFFECT OF SEASON ON WATER REQUIREMENT

Two significant results have been obtained in nearly all investigations of the use of water by crop plants: (1) Crop plants of different species show a marked difference in their efficiency in the use of water, and (2) seasonal conditions have a marked effect on the water require-

ment of a crop. These outstanding results have been confirmed in the experiments conducted by the writer.

In Table 32 the water requirement of alfalfa, wheat, millet, sorgo, and Sudan grass is shown (1) as actual values and (2) as index numbers in comparison with the evaporation from a free water surface during the period of most active crop growth, that is, June to August, inclusive. It is believed that the data obtained at Newell, S. Dak.,

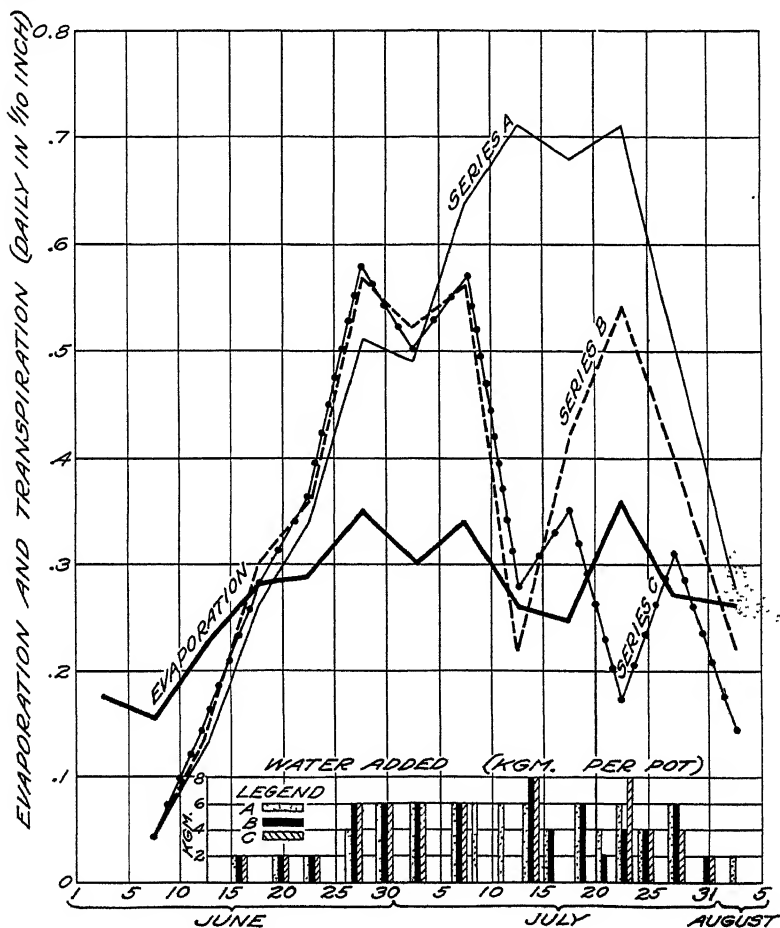


FIGURE 9—Mean daily transpiration (tenths of an inch based on the soil-surface area of the 16-inch pots) by 5-day periods of Swedish Select oats grown under different conditions of water supply at Mandan, N. Dak., in 1919: Series A, plants watered freely throughout the growing season; B, plants allowed to dry down and wilt on three or four successive days during the heading stage; and C, plants allowed to wilt during the heading stage and again during the late milk stage. Each line represents the mean of six pots, each pot containing from 18 to 20 plants. The heavy line represents the mean daily evaporation from a free water surface. The quantity of water in kilograms added to each pot and the date of watering are shown at the base of the graph. No water was added to series B and C from July 8 to 13 (6 days) nor to series C from July 15 to 22 (8 days).

and at Mandan, N. Dak., are comparable because the climatic conditions at the two stations are very similar. The average seasonal evaporation (April to August, inclusive) was 36.54 inches during the 17-year period 1908–1924 at Newell, and 34.21 inches during the 11-year period 1914–1924 at Mandan. The average seasonal precipitation was 9.07 inches at Newell and 10.13 inches at Mandan.

TABLE 32.—*Water requirement, based on dry matter, of field crops grown at Newell, S. Dak., and Mandan, N. Dak., 1912-1922, and evaporation from June to August, inclusive, expressed as actual values and as index numbers of the mean value for each crop, taken as 100; and ratio of water-requirement index to evaporation index*

SEASONAL WATER REQUIREMENT												
Data	Newell, S. Dak.						Mandan, N. Dak.					Average
	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	
Actual values:												
Alfalfa, all varieties <sup>a</sup>	701±7	759±6	1,036±14	602±5	694±5	898±5	947±10	700±8	670	936±33	742±11	802±10
Wheat, Kubanka, C. I. 1440	463±6	496±4	528±5	333±2	352±7	487±6	415±8	520±11	340±4	531±8	372±8	434±6
Millet, all varieties <sup>b</sup>	261±2	316±2	307±7	177±1	232±1	278±1	239±7	303±2	186±1			255±3
Sorgo, Dakota Amber				210±4	239±2	275±2	284±3	273±1	229±2			252
Sudan grass				272±2	314±5	344±5	333±3	347±4	274±3			314
Index numbers:												
Alfalfa, all varieties	87	95	129	75	87	112	118	88	83	117	93	100
Wheat, Kubanka, C. I. 1440	107	100	122	77	81	112	96	120	78	122	86	100
Millet, all varieties	102	124	120	69	91	109	94	119	73			100
Sorgo, Dakota Amber				83	95	109	113	108	91	100		100
Sudan grass				87	100	110	106	111	87			100
Average water requirement	99	106	124	78	91	110	105	109	82	120	90	
Ratio, WR/E	93	97	118	111	108	104	112	96	80	104	95	
Water-requirement index used	100	105	112	76	90	108	98	110	88	118	95	100
EVAPORATION, JUNE TO AUGUST												
Actual value (inches)	22.75	23.42	22.42	15.07	18.10	22.79	20.16	24.49	21.91	24.74	20.31	21.47
Index number	105	109	105	70	84	106	94	114	102	115	95	100

<sup>a</sup> The water requirement of alfalfa is based upon the mean value of three varieties (18 pots) grown in 1912, 1913, and 1914; six varieties (36 pots) in 1915 and 1916; five varieties (30 pots) in 1917; four varieties (24 pots) in 1918; and one variety (6 pots) in 1919, 1921, and 1922. The value for 1920 was computed.

<sup>b</sup> The value for millet is based upon four varieties (24 pots) grown in 1912; three varieties (18 pots) in 1913, 1915, 1916, and 1917; two varieties (12 pots) in 1914; and one variety (6 pots) in 1918, 1919, and 1920.

The use of index numbers was suggested by Shantz and Piemeisel (11) in order to facilitate the comparison of the water requirement of different plants with the evaporation from year to year. The annual index number is expressed as a percentage of the mean value taken as 100.

In the present paper the writer has used the evaporation for the 3-month period June to August, inclusive, as a basis of comparison with the water requirement, while Shantz used the 6-month period April to September, inclusive, in the report of his work at Akron, Colo. The shorter period is used here because it corresponds more closely to the period of active plant growth, especially in the area represented by these experiments.

The actual water requirement of the several crops during the 11-year period ranged as follows:

Alfalfa from  $602 \pm 5$  in 1915 to  $1,036 \pm 14$  in 1914.  
Kubanka wheat from  $333 \pm 2$  in 1915 to  $531 \pm 8$  in 1921.  
Sudan grass from  $272 \pm 2$  in 1915 to  $347 \pm 4$  in 1919.  
Millet from  $177 \pm 1$  in 1915 to  $316 \pm 2$  in 1913.  
Sorgo from  $210 \pm 4$  in 1915 to  $284 \pm 3$  in 1918.

The lowest value for each crop represented was obtained during the cool, wet season of 1915. In that year the remarkably low values of 177 for millet, 333 for wheat, and 602 for alfalfa were obtained. The highest value for each crop was obtained in different years.

The effect of seasonal conditions on water requirement can be seen readily by comparison of the index numbers, which, as already noted, are based on the mean value of each crop, with the evaporation taken as 100.

The evaporation index number varied from 70 in 1915 to 115 in 1921, a range of 45 points, while the average water-requirement index number varied from 78 in 1915 to 124 in 1914, a range of 46 points. It is evident, therefore, that the climatic factors which influence and determine the quantity of evaporation from a free water surface also determine the rate of transpiration from crop plants.

Considering only the 6-year period 1915 to 1920, when all of the five crops were grown, the evaporation index varied from 70 in 1915 to 114 in 1919, a range of 44 points; the water-requirement index of the several plants varied as follows: Alfalfa from 75 to 118, 43 points; wheat from 77 to 120, 43 points; millet from 69 to 119, 50 points; sorgo from 83 to 113, 30 points; and Sudan grass from 87 to 111, 24 points. The water-requirement range of alfalfa and of wheat was almost exactly the same as the range of evaporation, that of millet was somewhat greater, while the range of sorgo and of Sudan grass was only a little over half the range of evaporation. In other words, alfalfa, wheat, and millet are highly responsive to seasonal climatic conditions, while sorgo and Sudan grass are less responsive. The effect of seasonal conditions on evaporation and on the water requirement of the five crops mentioned above is shown graphically in Figure 10.

#### SEASONAL MARCH OF TRANSPIRATION

It is well known in the practice of irrigation that field crops use comparatively little water during the early stages of growth, when the plants are small, and that the rate of water use increases rapidly

as the plants approach their maximum development. It is of interest to compare the transpiration of crop plants grown in the water-requirement experiments with evaporation during short periods throughout the growing season. In Figure 11 the transpiration of Kubanka wheat is shown graphically as the mean daily loss in depth of water, based on the soil-surface area of the pots, in comparison with evaporation from a free water surface, that is, from a tank 8 feet in diameter set at the ground level. The graph is based on data obtained in 1915, a cool, wet season, and in 1917, a hot, dry season, at Newell, S. Dak.

In 1915 evaporation by 5-day periods ranged from 0.08 to 0.23 inch daily from June 10 to August 31. During this time the loss of water by transpiration from the six pots of wheat increased from 0.02 inch, for the period June 10 to 15, to a maximum of 0.27 inch daily, during the period July 25 to 31. This maximum was reached after heading. A progressive decrease then occurred until transpiration amounted to only 0.10 inch during the 5-day period before harvest on August 25.

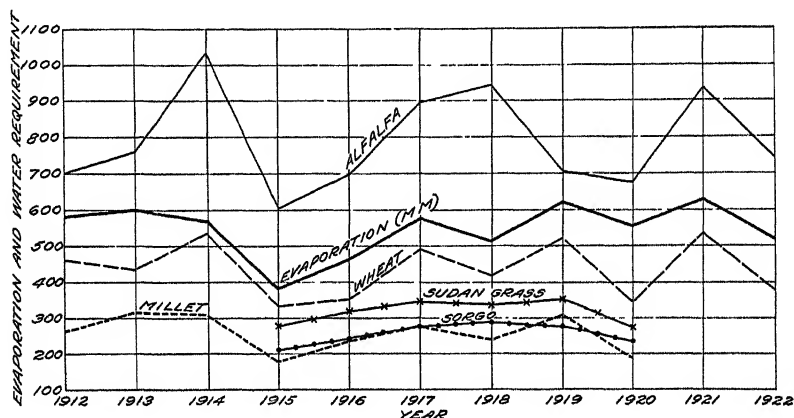


FIGURE 10.—Evaporation (June to August, inclusive) in millimeters and water requirement of alfalfa, wheat, millet, sorgo, and Sudan grass during the 11-year period 1912-1922

In 1917 both evaporation and transpiration were much higher than in 1915. (Fig. 11.) During July, evaporation exceeded 0.25 inch daily and reached 0.35 inch during one period, July 6 to 10. The daily loss of water by transpiration from wheat increased quite uniformly from the first period recorded, June 15 to 20, to the period after heading, July 21 to 25, when it amounted to 0.45 inch daily. After this period of rapid transpiration there was a progressive decrease in the rate of water use to the time of ripening. Transpiration during the last eight days before ripening averaged 0.12 inch per day as compared with 0.21 inch of evaporation.

The mean yield of total dry matter per pot was 129.6 gm. in 1915 and 113.6 gm. in 1917. It is evident, therefore, that the increase in rate of transpiration per pot in 1917 over that in 1915 was due to climatic factors rather than to a difference in leaf area, since the plants were somewhat smaller in 1917 than in 1915.

In Figure 12 the transpiration of Kubanka wheat at Mandan, N. Dak., in 1921 and 1922 is shown graphically in comparison with the mean daily evaporation by 5-day periods during the growing season.

The season of 1921 at Mandan was extremely hot and dry for that locality, and field crops were nearly a total failure. Maximum temperatures of 100° F. or higher occurred on several days in June and July, and 110° was recorded on July 9. The hot season resulted in the early ripening and comparatively low yield of wheat in the water-requirement experiments, the average dry weight per pot being 113.2 gm. in 1921 as compared with 214.6 gm. in 1922.

The daily transpiration of wheat exceeded evaporation of water from the tank during the period from June 20 to July 20, 1921, and from June 20 to August 10, 1922, both evaporation and transpiration being calculated on an equal water-surface or soil-surface area.

A growing crop is in fact a very efficient pumping system. In regions of limited rainfall, as on the Great Plains, a growing crop generally uses all the available water stored in the soil in addition to

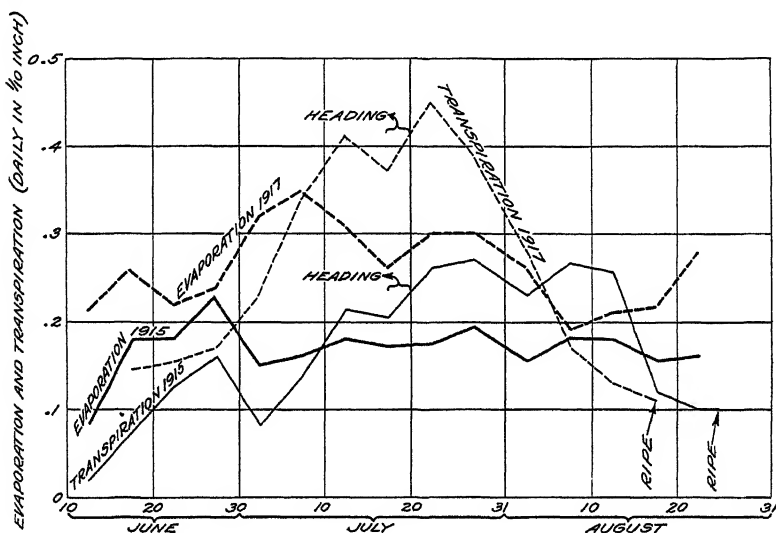


FIGURE 11.—Mean daily evaporation and mean daily transpiration of Kubanka wheat by 5-day periods at Newell, S. Dak., in 1915 and 1917. Transpiration is computed as depth of water transpired, based on the soil-surface area of the six pots of each crop grown. The progressive increase in the rate of transpiration of wheat as the plants approach the stage of greatest leaf development, usually some time after heading, followed by a progressive decrease in rate of transpiration up to ripening, is apparent.

that which falls during the growing season. According to Cole and Mathews (5), a crop of wheat on the northern Great Plains requires at least 4 inches of water under field conditions to mature any grain at all, and it will use as much as 16 to 18 inches if it is available. When the acre yield of wheat exceeded 10 bushels, it generally required from 640 to 1,150 pounds of water to produce 1 pound of dry matter. With lower yields the ratio of water used was generally much higher.

The possibility that a crop such as wheat, during its period of rapid growth, may pump more water into the atmosphere than is evaporated from a water surface of equal area has some interesting applications. In some of the drier States the impounding of water from artesian wells and from melting snow has been suggested as a means of increasing evaporation and, supposedly, the summer rainfall. It is probable, however, that the soil itself is the most practicable storage reservoir



and the growing crop the most efficient pump to set the water free. In wet seasons, when the growth of native vegetation and of cultivated crops is luxuriant, to what extent does transpiration of vegetation contribute to the supply of atmospheric moisture which may be precipitated as more or less local showers? When soil moisture is readily available, it is possible that the transpiration of native vegetation during the early summer may equal evaporation of water from an equal area. Under irrigation it is certain that most crops in full leaf transpire more water than would be evaporated from an equal area of water surface.

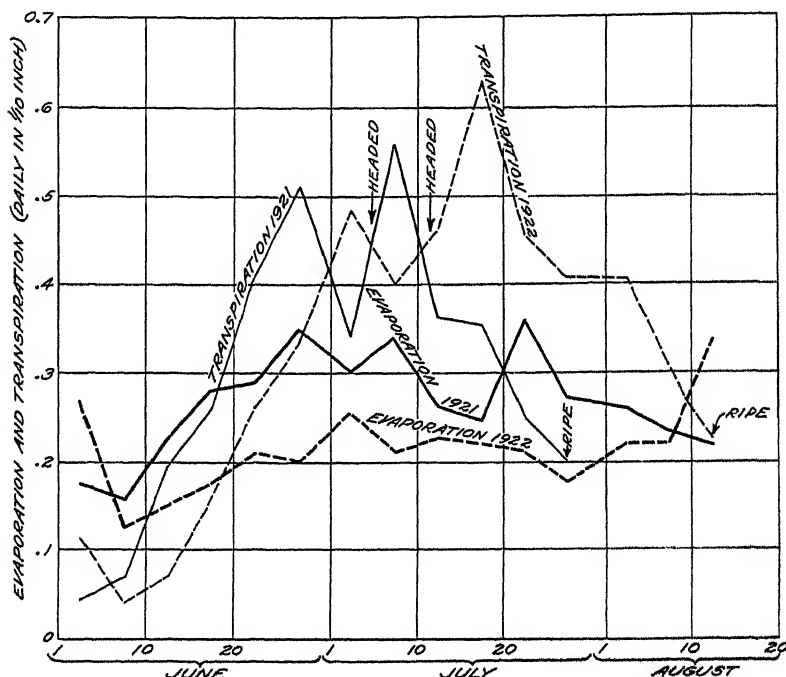


FIGURE 12.—Mean daily evaporation and mean daily transpiration of Kubanka wheat by 5-day periods at Mandan, N. Dak., in 1921 and 1922. The higher rate of transpiration in 1922 is due to the larger plants, the final dry weight of plants per pot in 1922 being 214.6 gm. as compared with 113.2 gm. in 1921. Transpiration exceeded 0.6 inch per day at the maximum (July 16-20, 1922) when, during the same period, evaporation from the tank did not exceed 0.25 inch per day.

#### RELATION OF SEASONAL CLIMATIC CONDITIONS TO YIELD AND WATER REQUIREMENT OF WHEAT

It is of interest to observe the relation between seasonal climatic conditions, as measured by evaporation, and the yield and water requirement of wheat. Figure 13 shows graphically the yield of Kubanka durum wheat after summer fallow, the seasonal (April to July) precipitation, the reciprocal of the index of evaporation, and the reciprocal of the water requirement of wheat at Newell, S. Dak. (1912 to 1918), and at Mandan, N. Dak. (1919 to 1922). In 1915, 1916, 1918, and 1922 the yield of wheat was at or above the mean (18.6 bushels per acre) when evaporation and water requirement were well below the mean (the reciprocal is shown for more ready compari-

son). The years 1914, 1917, 1919, and 1921 were seasons of high evaporation and of high water requirement and low yields. In 1912 the crop failure was due to extreme drought during June. In 1920 drought prevailed during June and the greater part of July, and in 1921 the low yield was due to high temperatures and drought in May and June.

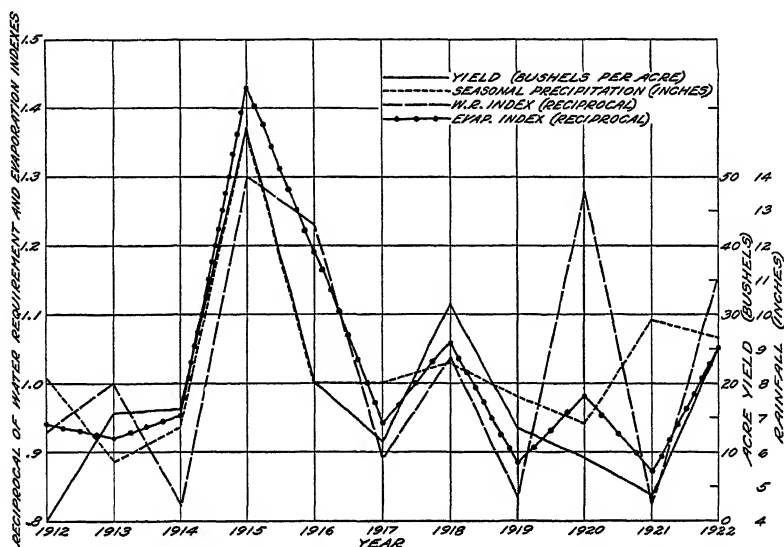


FIGURE 13.—Relation between climatic factors of evaporation and seasonal rainfall to yield and water requirement of Kubanka wheat. The yield is based on the average annual yield of five  $\frac{1}{10}$ -acre plots at Newell (1912 to 1918) and of 12 plots at Mandan (1919 to 1922). All plots were fallow and clean cultivated the year previous to cropping. The seasonal precipitation is for the four months, April to July. Evaporation is for the three months, June to August, for better comparison with the water-requirement determinations, in which the wheat generally was later than in the field plots. Evaporation and water requirement are expressed as the reciprocal of the index number. (Table 32.) In 1915, 1918, and 1922 rainfall was above normal and evaporation was below normal, resulting in a high efficiency of transpiration (low water requirement), whereas in 1914, 1917, 1919, and 1921 climatic conditions were unfavorable, resulting in low yields and a high water requirement. The low yield of 1920 was due to drought in June and the greater part of July.

### SUMMARY AND CONCLUSIONS

A summary of the comparable water-requirement determinations made during the 11-year period covered by the experiments is presented in Table 33. The work was conducted at Newell, S. Dak., for seven seasons, 1912 to 1918, and at Mandan, N. Dak., for four seasons, 1919 to 1922. A total of 110 sets of plants, or about 660 individual pots, was grown during the investigations.





As many of the crops were grown for only a part of the period, some correction must be made for the character of the growing season if the mean values of different crops are to be compared. This has been done in the column headed "Weighted mean" which is based on the "water-requirement index used" as shown in the last line of Table 32. For example, the weighted mean value of Kubanka wheat is the mean of the annual values each divided by the "water-requirement index" of that year. The actual mean value for Kubanka wheat was 432 and the weighted mean was 430. On the basis of the weighted mean it is possible to compare the water-requirement values of the different crop plants and weeds represented. The lowest values, based on dry matter, were found in the millets and sorgos and the highest values in the alfalfas and grasses. In the last column is shown the "efficiency of transpiration," a term used by Maksimov (7) to express the number of grams of dry matter produced from 1,000 gm. of water transpired.

The range of water requirement is very wide, extending from 224 for Russian thistle to 1,183 for western wheatgrass. The lowest actual value obtained from any crop was  $171 \pm 1$  for Kursk millet, A. D. I. 3, in 1915.

As mentioned above, Kubanka wheat had a weighted mean water requirement of 430, or an efficiency of transpiration of 2.33. Three varieties of common wheat each had a somewhat higher water requirement than Kubanka durum wheat in 1922, the only year they were grown. Swedish Select oat, grown only two seasons, had a weighted mean value of 536 or a transpiration efficiency of 1.87.

Kursk millet, A. D. I. 3, grown for nine seasons, had a weighted mean water-requirement value of 251. This value was considerably lower than that of Kursk, A. D. I. 13-3, a sib selection (280), but about the same as that of the Siberian (259) and Gold Mine (253) varieties. Apparently Red Amber sorgo (253) was slightly more efficient in the use of water than Dakota Amber sorgo (268), though this difference may have been due to maturity, Dakota Amber usually maturing a normal yield of seed, while Red Amber did not except for a small yield in 1916. Sudan grass is much less efficient in the use of water than millet or sorgo. Its water requirement was 335, or about 25 per cent higher than that of Dakota Amber sorgo.

The perennial grasses—brome grass, crested wheatgrass, and western wheatgrass—gave weighted mean values of 784, 853, and 1,183, respectively. These values are probably too high as compared with the real efficiency of the grasses as dry-land crops. All are perennials, and during late summer under normal conditions they store organic food material in their rhizomes and roots, which enables them to produce an early summer crop the following year with probably an efficient use of water. (See Table 17.)

The annual weeds—witch grass, Russian thistle, redroot pigweed, and purslane—had low weighted mean values, ranging from 224 for Russian thistle to 288 for purslane, as compared with 435 for lamb's-quarters. The Russian thistle is remarkably efficient in the use of water, and this probably explains why it is such a serious weed pest in dry seasons. Sugar beet grown only one season had a water requirement of 304, based on total dry matter of roots and tops.

The actual mean water requirement of six varieties of flax ranged from 738 to 902. The values obtained in 1922 were abnormally

high, in spite of the favorable growing season, due to the fact that (in the writer's absence) the soil was kept too moist during the ripening period, thus stimulating late vegetative growth after the seed bolls were mature. This effect was not so evident in the early variety, N. D. R. 114, which had a lower value in 1922 than in 1921. The average weighted-mean value of 668 for all varieties probably is near the true value under normal conditions at Mandan.

Yellow-flowered alfalfa (*Medicago falcata*) had a weighted mean water requirement of 702, as compared with 798 as the average weighted mean value of varieties of *M. sativa*. *M. falcata* shows a remarkable difference in the water requirement of successive crops. In 1913 (Table 16) the first crop, harvested June 30, had a water requirement of  $340 \pm 7$ ; the second crop, harvested August 6, had a value of  $713 \pm 11$ ; and the third crop, harvested September 18, had a value of  $1,220 \pm 52$ . These pots were planted in 1912. Very similar results were obtained with Grimm alfalfa when the plants were overwintered. In 1913 (Table 13) the three crops of Grimm, A. D. I. E-23, harvested on the same dates as *M. falcata*, gave a water-requirement value for the early-summer crop of  $469 \pm 6$ ; for the midsummer crop,  $817 \pm 13$ ; and for the late-summer crop,  $1,115 \pm 16$ . Alfalfa is in fact quite a dependable crop in dry-land farming on the northern Great Plains, although in most seasons it produces only a single early-summer crop. This one crop, as shown above, probably is produced with a water requirement only a little higher than that of wheat.

The results of the experiments, although not conclusive, throw some light on the problems suggested in the introduction, namely:

(1) Whether the water requirement can be used as a measure of the relative adaptation to conditions of drought of different varieties of the same crop. The data available indicate that there are small but significant differences in the water requirement of different varieties of the same crop. However, as the water-requirement value in pot experiments as usually conducted is determined under conditions of adequate soil-moisture supply, it probably is not a dependable measure of the adaptation of a variety to conditions of drought. A low water requirement suggests adaptation to drought, but the final measure of the value of a variety must be the actual yield as determined by carefully conducted field-plot tests.

(2) Whether similar varieties or selected progeny strains show appreciable differences in water requirement. The water requirement of related strains of millet and of alfalfa did not show significant differences over a period of years. (Table 33.) As pot experiments are subject to errors of experimentation, especially in any factors which affect the yield of the plants, it is doubtful whether the water requirement can be used as a precise measure of the value of related strains. In nearly all experiments with millets the probable error based on six pots of each variety was very low; whereas, with alfalfa, the probable error was comparatively high. Although the methods of experimentation in water-requirement determinations may not be refined enough to measure small differences between selected strains of a variety, or between similar varieties, they do indicate the great range of values of different crop plants. It is this knowledge of the water requirement of different crop plants and weeds which is of significant practical importance.

(3) Whether the water requirement of crop plants is correlated with yields as determined by plot tests in the field. In the case of Kubanka wheat there was in most years a significant relation between the water requirement and the yield of grain in the field. This subject is discussed under the topic "Relation of Seasonal Climatic Conditions to Yield and Water Requirement of Wheat."

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# THE TOXICITY OF CORYDALIS CASEANA<sup>1</sup>

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## INTRODUCTION

Serious losses of sheep on certain portions of summer range in the Plumas National Forest, Calif., directed suspicion to a succulent plant growing in some of the canyons of the range. The owner of the flocks submitted samples of the suspected plant, which was identified as *Corydalis caseana* Gray. This identification was later confirmed through the courtesy of the Forest Service of the United States Department of Agriculture. The plant is also referred to as *Capnoides caseana*. Because of the effect of the plant on sheep the name "fit weed" has been suggested for common use.

Fresh material collected in July, 1929, was used in feeding experiments with sheep at this station. These tests demonstrated conclusively that the plant is toxic. The results of these tests will be presented as a publication of this station from the department of range management. Material collected in 1929 and 1930 was used in the work with rats herein reported.

## BOTANICAL DESCRIPTION OF CORYDALIS CASEANA

*Corydalis caseana* Gray belongs to the order Fumariaceae and is described (8, p. 96, 467)<sup>3</sup> as follows:

*Caseana*, GRAY. Stem rather lax and succulent: hood of the outer petals concave, with spreading margins, pointless or short-pointed, and bearing a rather broad and apically projecting dorsal crest: mature fruit unknown, the forming capsules barely half inch long, elliptical, obtuse.—Proc. Am. Acad. X. 69; Wats. Bot. Calif. ii, 429. *C. Bidwellia*, Wats. l. c.—In water or on very wet banks, Sierra Nevada, California, from Truckee River to the Big Spring district in Plumas Co., Bolander, E. L. Case, Lemmon, Mrs. Austin, Mrs. Bidwell, Parry.

*C. Caseana* Gray, p. 96. Add syn. *Capnoides Caseanum*, & *C. Bidwellianum*, Greene, l. c. 280.

## PREPARATION OF THE CRUDE ALKALOIDAL MATERIAL

So far as the available literature discloses, no chemical investigation of *Corydalis caseana* has been reported. However, notable researches on the alkaloids of the bulbous roots of *C. cava* have been made by Freund and Josephy (7), Dobbie and Lauder (1, 2, 3, 4, 5, 6), and others. The aboveground portions of *C. cava* have been but little studied, although Haars (9) reports finding bulbocapnine in the tops, and also two other alkaloids. He did not, however, find corydaline in the tops, a constituent which is present in greatest quantity in the roots. Protopine, which has been reported as occurring in the roots, he found to be lacking in the aboveground parts.

<sup>1</sup> Received for publication Dec. 4, 1930; issued February, 1931.

<sup>2</sup> Grateful acknowledgment is made of the assistance in parts of this work given by Dr. Lyman R. Vawter, of the veterinary department of this station.

<sup>3</sup> Reference is made by number (italic) to Literature cited, p. 243.



A portion of the fresh green plants collected in July, 1929, was received at the laboratory in good condition. A sample, when dried at ordinary room temperature lost 92.7 per cent of its weight; the material collected in July, 1930, lost 91.8 per cent. These figures indicate the succulence of the plant.

Preliminary work on the fresh green plant was undertaken to determine the presence of alkaloids. For this purpose, the green plant tissues were broken up and allowed to stand 24 hours with an equal volume of 95 per cent alcohol. The alcoholic extract was removed by pressure, concentrated to a sirup, mixed with purified sawdust and dried. Successive extractions were made on the sawdust mixture, using petroleum ether, benzol, and chloroform. Each of these solvents yielded material that gave positive reactions with the general alkaloidal reagents, after their removal with dilute sulphuric acid and recovery by extraction with ether. The alkaloidal reagents used were Dragendorff's, Mayer's, and Wagner's, and silicotungstic acid.

The plant residue, after removal of the alcoholic extract and washing, was air-dried, ground, and extracted with a dilute aqueous solution of tartaric acid until the liquid from the percolator was colorless. The extract was then concentrated to approximately one-fifth its volume, the precipitated material removed, and the clear liquid extracted with ether. The material removed by the ether gave no indication of the presence of alkaloids with the alkaloidal reagents. The acid aqueous extract was then made distinctly alkaline with sodium hydroxide and again extracted with ether. After removal of the ether a small residue remained, which gave positive reactions with the alkaloidal reagents.

A small portion of air-dried and ground plant was extracted in the cold with 95 per cent alcohol, the extract concentrated to a thick sirup, and treated with dilute acetic acid. The yellowish-brown aqueous solution so obtained, made alkaline with ammonium hydroxide and extracted with ether, yielded, after drying, a sirupy residue amounting to 0.85 per cent of the dry plant used. After this residue had stood for several days a very small amount of crystalline material separated. The crystals and mother liquor were taken up with dilute hydrochloric acid and after having been made alkaline were again extracted with ether. The solid material obtained from the ethereal solution was again treated in the same manner and finally yielded a very small quantity of crystals in spherical masses, which, when further purified by recrystallization from ethyl acetate, melted at 199° C.

Determinations of the content of crude alkaloids were made on the air-dried plant by the ordinary method. Ten-gram samples were macerated with Prollius's fluid for 24 hours, the extracts removed by suction, and the residues washed with alcohol. The filtrates were shaken out with several portions of dilute sulphuric acid, the acid aqueous extracts made slightly alkaline with sodium carbonate, and extracted with ether in several portions. The ethereal extracts were colored yellow. After washing and drying, the solvent was removed, and the residues were dried over sulphuric acid and weighed.

The assays showed that the air-dried plant which had been collected in 1929 contained 1.10 per cent of crude alkaloids, and that collected in 1930, 1.90 per cent. The crude alkaloidal residue, when dry, con-

sisted partly of a nearly white granular material and partly of a yellowish-brown, varnishlike mass. When treated with dilute hydrochloric acid and warmed, the residues were nearly completely soluble, giving yellowish-brown solutions and leaving small quantities of dark-gray resinous matter. The solutions so formed gave positive reactions with the alkaloidal reagents.

#### PHYSIOLOGICAL TESTS

An effort was next made to determine the toxicity of the crude alkaloidal residues from the material gathered in 1930. They were first dissolved in a small quantity of very dilute hydrochloric acid and the solutions made to volumes of 10 c. c. A rat weighing 200 gm. was then injected subcutaneously with 0.50 c. c. of one of these solutions. After 10 minutes the animal became markedly depressed and moved about very slowly when not remaining quiet. It continued in this condition for approximately 3 hours but gradually became as active as before the injection. Observation for several days gave no indication of further symptoms. Another 200-gm. rat injected subcutaneously with 0.25 c. c. of the same solution showed the same quieting effect in about 10 minutes. Unlike the first animal, this rat began to exhibit clonic convulsions after 15 minutes, and these increased in severity until it died 45 minutes after the injection. Post-mortem examination showed dilation of the heart and congestion of the lungs. Moderate congestion of the upper part of the small intestine was also observed, with traces of blood in the intestinal contents. The heart had stopped in systole. A striking cyanosis of the mucous membrane of the mouth was noted. Death had resulted apparently from respiratory failure. Why the smaller dose should have been more toxic than the larger is not clearly understood.

A duplicate preparation, similar in all respects to the one used above, except that it had been allowed to stand for 24 hours, was next used. Two 200-gm. rats were each injected subcutaneously with 0.25 c. c. of this solution. Both showed the sedative effect in 15 minutes, remained quiet for approximately 3 hours, and recovered. Two 200-gm. rats were similarly injected with 0.50 c. c. of the preparation. In 15 minutes both became quiet. After 30 minutes one had intermittent mild convulsive seizures for a period of approximately an hour, after which it gradually became more normal, and after 3 hours both were more active and finally recovered. A 200-gm. rat was injected with 0.25 c. c. of the same solution as that used in the first test, 24 hours after its preparation. In 20 minutes the rat became quiet, remained in this condition for nearly 3 hours, and then made a rapid recovery.

The result here noted, namely, the greater action of the more recently prepared solution, called to mind the work reported by Dobbie and Lauder (1). These investigators state that corydaline, obtained from crude corydaline as supplied to them by Schuchardt and prepared from the roots of *Corydalis tuberosa*, is sensitive to light and heat. According to their observations, light and heat produce a change in corydaline, resulting in a yellow alteration product which is without the properties of the original substance.<sup>3</sup>

<sup>3</sup> A similar alteration in nicotine solutions after exposure to the mercury vapor lamp has been reported by A. J. Pacini and McGuigan. (10).

With this in mind the writer made new preparations, using the same quantity of dry plant (10 gm.) and the same method. All of the work, however, was carried out in subdued daylight, sufficient only for convenience in manipulation. The solutions in very dilute hydrochloric acid were prepared by warming, as before, except that they were exposed to the heat of the water bath for a shorter period than formerly. The volume of the solutions was 10 c. c. as above. After preparation, the solutions were kept in a light-proof container until used. The solutions were administered as soon as possible after preparation.

A freshly prepared solution of crude alkaloidal residue which had been separated in subdued light was injected subcutaneously in increasing doses into rats weighing 200 gm. Administration of 0.25 c. c. produced a sedative effect only; 0.35 c. c. produced a quieting effect followed by intermittent spasms, with recovery; 0.40 c. c. and 0.50 c. c. produced quieting, then convulsions, and 30 minutes after injection, death. Autopsy of the rats killed in this series of tests showed changes similar to those described above.

In all the work the same concentrations were used. The very dilute hydrochloric acid solution was equivalent, per cubic centimeter, to 1.0 gm. of air-dry plant, 12.2 gm. of fresh plant, and 0.0190 gm. of crude alkaloids.

Based on the results obtained by using alkaloidal material prepared in subdued light, the lethal dose for 200-gm. rats (0.40 c. c.) is equivalent to 0.0076 gm. of crude alkaloids, or 4.88 gm. of fresh plant. This is equivalent to 24.4 gm. of fresh plant per kilogram of body weight.

### CONCLUSIONS

That *Corydalis caseana* is highly toxic is shown by the results given above. From the results reported in the literature, plants belonging to this family appear to be rich in alkaloids. It is presumed that in *C. caseana* there exists more than a single alkaloid, and this point is being further studied at this station.

### SUMMARY

Serious losses of sheep on certain portions of summer range in the Plumas National Forest, Calif., directed attention to a plant identified as *Corydalis caseana* as the possible cause of death. Chemical examination of the plant resulted in the preparation of from 1.10 to 1.90 per cent (on a dry basis) of crude alkaloidal material therefrom. Physiological tests demonstrated the toxicity of the plant. The lethal dose for 200 gm. rats was equivalent to 0.0076 gm. of the crude alkaloids, or 24.4 gm. of fresh plant per kilogram of body weight.

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# AN ANALYSIS OF REPUTED PATHOGENICITY OF THYSANOSOMA ACTINIODES IN ADULT SHEEP<sup>1</sup>

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## INTRODUCTION

Since the work of Curtice (1)<sup>3</sup> there have been numerous reports concerning the pathogenicity of the fringed or fimbriated tapeworm, *Thysanosoma actinioides* (*Taenia fimbriata*) Dies., 1834. Ofttimes these reports, especially more recent ones, are contradictory regarding symptoms and diagnosis. "Fimbriata" infection, like hemorrhagic septicemia, is all too often a convenient blanket term to cover various ailments of sheep.

Very often loco and silvery lupine poisoning have been confused with the tapeworm infections. Curtice (1) considered the condition of many so-called locoed sheep due to this parasite. Ward (8, p. 288) states: "It seems probable that the loco of the western sheep has for its exciting cause this or a similar form." Marshall (5), on the other hand, examined 11 locoed sheep and found 9 of them harboring the fringed tapeworm. Durrell and Glover (2) found chronic lupine poisoning more common in sheep than loco, the latter being more prevalent in horses. They found that lupine poisoning is often associated with liver disorder and jaundice. It is not the purpose of this paper to consider the relationship of tapeworm infection to poisoning but to point out that, contrary to popular belief, *Thysanosoma* infection is rarely lethal to adult sheep.

Attention was directed to the problem by the fact that recently in certain feed lots in Minnesota excessively high mortality occurred, amounting to weekly losses of 14 sheep per thousand. Post-mortems by veterinarians revealed *Thysanosoma actinioides* in the liver and bile passages. The losses were attributed to this parasite, although the symptoms were atypical. The explanation was that the presence of the tapeworms in the bile and pancreatic ducts prevented the flow of these juices into the small intestine. Since these essential juices were lacking the flow of food from the stomach was immediately closed off. The stomach would thus be full of food in a fermenting condition, and the animal would die of autointoxication. A copper sulphate treatment was advised. This supposedly caused many of the sheep to recover and presumably removed the worms from the intestine and the liver with equal efficacy. This was regarded as substantiated by port-mortems on three sheep, all of which were found negative.

This theory of the rôle of *thysanosoma* being accepted, the writer was asked to take up the study of the life cycle of the worm with a

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<sup>2</sup> To Swift & Co., at South St. Paul, and particularly to Carl Elmer, chemist in charge, the writer wishes to express his obligations for aid and ready cooperation in all phases of the work. He wishes also to acknowledge his indebtedness to C. H. Easterlunt, who aided in making the examinations.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 249.

view to developing control measures. Early in the work doubt arose as to the validity of the previous conclusions and it was decided to study the incidence of the thysanosomes in sheep presenting symptoms of succumbing to the disorder.

### RESULTS OF EXPERIMENTAL EXAMINATION OF SHEEP

Doubts first arose in connection with the records of mortality among some 30,000 sheep over a 2-year period. As shown in the curve, the greatest losses occurred toward the end of the feeding period. (Fig. 1.) Figure 2 shows the normal death rate of sheep under feed-lot conditions. Providing chances for reinfection are not

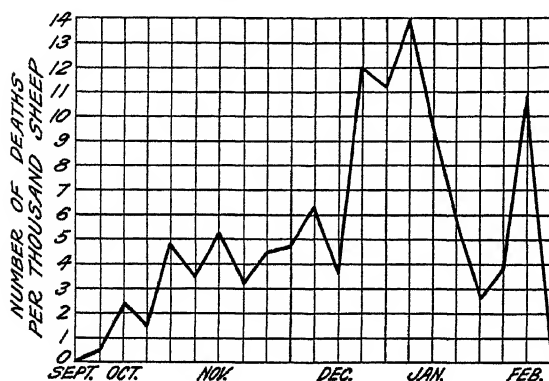


FIGURE 1.—Excessive mortality of sheep beginning two and one-half months after the animals were placed on feed; the peak occurred during moderate winter weather, the low points during colder weather

present, one would expect the peak of the curve near the beginning of the feeding period when the most heavily diseased animals would die. Again, one would expect the highest mortality when the weather conditions were adverse as pointed out by Curtice (1) and Ward (8). Upon this basis the losses were charted against the temperature.

During the subzero weather of January the mortality curve approached the base line, and during the abnormal moderate weather of December the peak in the curve was reached.

In a preliminary examination to determine the extent to which the fringed tapeworm might be incriminated, 12 sick sheep, which presented typical symptoms of the prevailing disorder, were selected from the isolation pen. Some were on the verge of collapse. All had diarrhea, a certain nervousness expressed by shaking of the head, but were possessed of good quality in wool and flesh. Postmortems showed that four were infected with the tapeworm, but that only one had sufficient numbers to clog the bile ducts materially. Yet all presented typical symptoms, and some were down prior to the time the examination was made. None showed evidence of jaundice.

The next 250 sheep showing typical symptoms, many on the verge of collapse, were killed and examined. The result was astonishing. Here the rate of infection by *Thysanosoma actinioides* was but 4.1 per cent. This slight incidence in the same lot of sheep examined

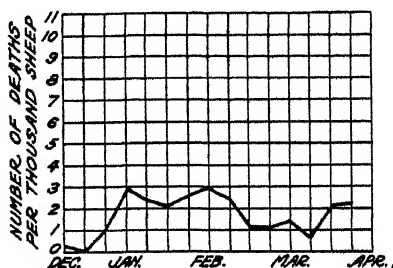


FIGURE 2.—Normal mortality curves of sheep under feed-lot conditions for comparison with Figure 1; here also fluctuations correlate closely with temperature values

by the veterinarian could readily account, upon a chance basis, for the three uninfected animals treated with the copper sulphate, as previously mentioned. A physical examination of the 10 sheep represented by the 4.1 per cent showed little intestinal inflammation, no appreciable glandular enlargement, and the meat to be of excellent quality and the wool in perfect condition. Indeed these sheep were among the biggest and fattest in the lot, and did not at all appear as if infected by an insidious disease.

These studies led to the examination of 253 apparently normal sheep for comparison. In this lot 6.7 per cent were infected, or a 2.6 per cent higher rate than in those presumably dying from the tapeworm. This was subsequently checked by the examination of 500 more apparently normal animals, from the same pen containing the sick sheep, and a 5 per cent infection was noted, this again being higher than in the ailing sheep.

In making the examinations only the liver and the bile ducts were examined. This was done on the assumption that it is in these localities that the lethal action occurs. To render the above statistics more valid it must be shown that there was no migration of the worms into the intestine after the death of the animal and that the liver infection is really an index to the rate of intestinal infection. This was done in the following manner:

Post-mortems were performed on 100 apparently normal sheep, they being first examined for hepatic infection, and then checked for intestinal incidence. Of these, 20 per cent showed infection, 15 per cent having parasites in the liver and intestine and 5 per cent in the liver alone. Then a similar lot of 125 apparently normal sheep was examined and a 74 per cent infection found. These sheep were shipped from Colorado, where the parasite is reported to be most common. In these the intestinal infection was checked first, with subsequent liver examination. Eight animals had hepatic infections alone, 50 had intestinal infections, and 35 had coincident infections of the liver and the intestine. It is thus clear that although the intestine is the primary habitat of this tapeworm, it also is found quite commonly in the liver. It seems that a concentrated bile content is essential for the parasite, inasmuch as it is found only in the upper part of the duodenum, the liver and bile ducts, and rarely in the pancreatic ducts.

#### DISCUSSION

It has been assumed that the pathogenicity of thysanosomes was produced by clogging the bile passages. In the last lot of sheep examined a few presented traces of jaundice in the conjunctivae and the subcutaneous tissues. It was decided to study the extent of bile stoppage in such animals. To demonstrate this the sheep having a slight jaundice because of extremely heavy infection were tested for bile, using both Gmelin's and Kettendoller's tests. All were positive for bile in both the duodenum and the feces.

These studies show that the fringed tapeworm did not cause the losses in the present case and that this diagnosis resulted from the chance finding of worms commonly regarded as lethal. To recall the recorded symptoms associated with this parasite we refer to Curtice and Hall.



Curtice (1) reports that lambs infected with thysanosomes are large-headed, with undersized bodies and hidebound skins. Their gait is stiff, and they have difficulty cropping the shorter grass. Others do not see well, and apprehend danger poorly. The liver is smaller, the kidneys flabbier and paler, the lymphatics somewhat darker and the muscles thinner and weaker than in normal animals. In all cases there is leanness of muscle and a diminution of fat. The symptoms and pathogenic lesions are those of malnutrition. These conditions prevail in both adults and lambs in heavy infections.

Hall (3) reports that the obstruction of the bile ducts causes inflammation of these ducts. The parasite causes alteration of secretion, with digestive disorders as a consequence. An unthrifty condition is produced which shows in poor quality of flesh and wool. Infected sheep are liable to die during adverse weather conditions and are commonly hidebound and suffer from diarrhea.

On the other hand, Jungherr and Welch (4) find stiff gaitedness due to three infections: (1) Umbilical infection, (2) coccidiosis, and (3) *Vibrio septique* infection. Welch (9) reports the fringed tapeworm as common in the bile ducts of young lambs and old ewes in Montana, but is unable to attribute actual damage to it. This same attitude is taken by Newsom and Cross (6), who consider this tapeworm of little significance.

In the earlier part of this paper reference is made to the use of copper sulphate for removing the worms from the affected sheep. All authorities agree that medication is ineffective, removing at best only worms located in the intestine. Curtice (1) tried all of the standard taeniafuges with no success. Ward (8) discourages treatment on the basis that drugs used to kill the parasites in the liver would undoubtedly have a serious effect upon the host. Hall (3) states that no successful treatment is known, those used having failed, and that the best recommendations for infected animals are careful nursing and good feeding.

Stiles (7) in summarizing the distribution of *Thysanosoma actinioides* lists the following countries and States: Brazil, Colorado, Utah, Nebraska, New Mexico, California, Oregon, Missouri, and Washington, D. C. (imported from Colorado). Hall (3) finds the eastern limit to be North Dakota, South Dakota, Nebraska, Kansas, Oklahoma, and Texas. W. A. Riley records the occurrence of *T. actinioides* in Minnesota, in addition to this range. Of 30 native Minnesota sheep examined by the writer 5 were found infected with this parasite.

#### SUMMARY

The symptoms associated with infection with *Thysanosoma actinioides* are often confused, and therefore it has been given undue pathogenic importance. Post-mortem studies of over 1,200 sheep here reported have shown that in well-organized feed lots no great damage is done to the host except where excessively heavy infections occur. In cases of heavy infection the symptoms simulate those of malnutrition, and this fact should be given due weight before the mortality is attributed to the presence of a few thysanosomes in the liver. The conditions prevalent in the ailing sheep examined were fairly similar to those of sheep suffering from apoplexy as described by Wing (10, 11), or from what Newsom and Cross term "overeating." Unlike

the work of the latter writers, however, the author's studies show but few animals to have the extensive hemorrhagic areas which they figure.

The liver appears to be part of the normal habitat of the parasite. Whether the migration to that locality is cyclic or not can not be said, but it is evident that the parasite occurs there almost as frequently as in the intestine.

Even where the liver infection is most heavy, analyses show that bile is present in both the duodenum and feces. Undoubtedly, as Curtice holds, the flow is not of normal regularity. For that matter a sheep's feeding is not periodic in feed lots, and much of the bile could function in a normal manner.

The area in which *Thysanosoma actinioides* infection occurs is gradually increasing. Minnesota sheep are infected as well as those from regions previously recorded. In spite of this gradual spread, and the prevailing idea of its pathogenicity in growing lambs, nothing is yet known regarding the life cycle of *T. actinioides* except that it probably requires an intermediate host.

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# JOURNAL OF AGRICULTURAL RESEARCH

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## GENETIC FACTORS FOR PIGMENTATION IN THE ONION AND THEIR RELATION TO DISEASE RESISTANCE<sup>1</sup>

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### INTRODUCTION

The common onion (*Allium cepa* Linn.) has been used extensively in studies on the nature of disease resistance. It has been demonstrated that, in general, pigmented onion bulbs are highly resistant to the onion smudge organism (*Colletotrichum circinans* (Berk.) Vogl.) while pigmentless bulbs are susceptible. This relationship between parasite and host affords a rather unique opportunity for fundamental research on the nature and inheritance of disease resistance. Although many cases of disease resistance in plants have been reported, in no other instance, so far as the writer is aware, has a specific visible character been recognized which is definitely associated with a lethal effect on the parasite. Not only are the pigments located in the outer scales of the bulb known to be associated with toxic materials, but a toxic chemical entity has been isolated from pigmented scales that is not present in pigmentless scales (5, 12).<sup>3</sup>

Tschermak (8) reports that dark yellow and red bulb colors are dominant to white in the  $F_1$  generation. In the  $F_2$  generation a complex segregation was observed, and in the  $F_3$  generation some white individuals produced white and colored bulbs. He observed that the so-called white commercial varieties of onions produce many plants showing light-red or yellowish pigmentation. The data presented in this paper also show that the yellow and red bulb colors are dominant to a recessive white in the  $F_1$  and later generations. However, a dominant white was also noted, which readily accounts for the complex segregation Tschermak encountered in the  $F_2$  and  $F_3$  generations resulting from open-pollinated insect crosses.

The nature of the interaction of color factors which determine the physiological expression of chemical entities responsible for resistance found in the outer scales of the onion form the basis for this study.

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<sup>2</sup> It is with gratitude that the writer acknowledges the valuable advice and assistance of Dr. R. A. Brink and Dr. J. C. Walker, who have made this study possible. The writer is indebted to Doctor Walker for the photomicrograph of Plate 3.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 277.

## DESCRIPTION OF THE SMUDGE DISEASE

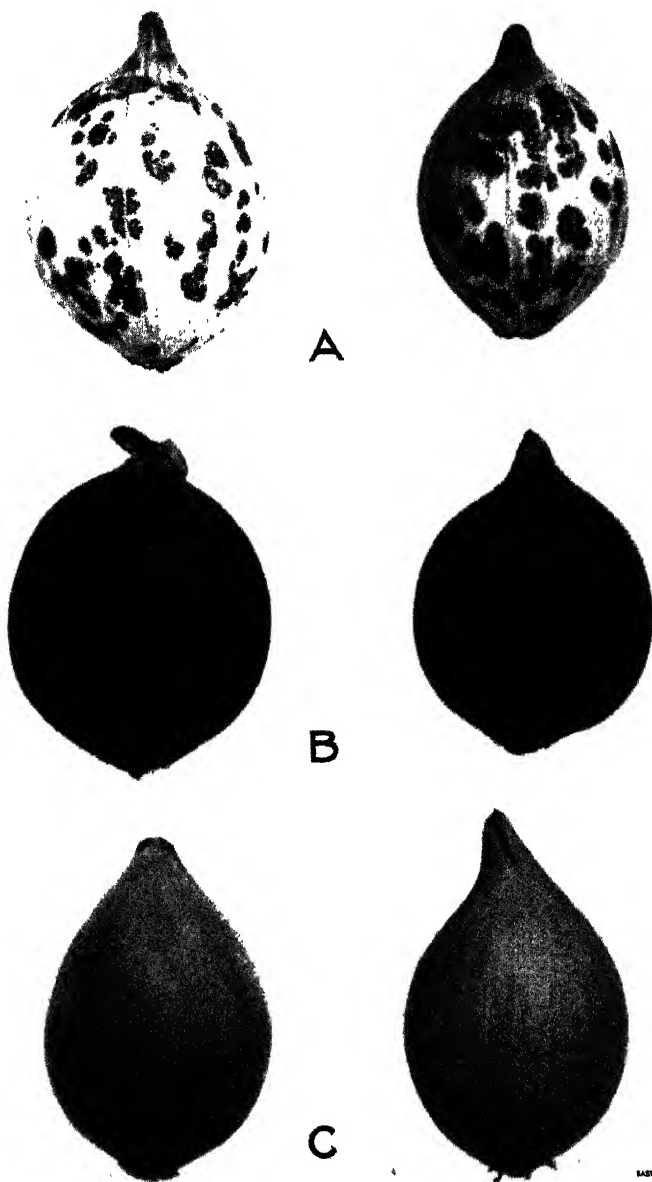
Smudge occurs on the common onion, shallots, and leeks, causing a marred appearance, shrinkage of bulbs, and premature sprouting. The disease may develop at any time during the life of the plant. The most common symptom is the dark-green or black smudge on the bulb or neck of the onion. (Pls. 1 and 2.) In some cases the surface of the lesion may be uniformly black, but more commonly the stromata and visible mycelium of the parasite are arranged in concentric circles. In severe cases the fungus grows through the outer dry scales into the turgid living tissue, causing a collapse of the fleshy scales. On pigmented onions the fungus is confined almost entirely to the neck of the bulb, where the flattened leaves are colorless.

## RELATION OF THE PARASITE TO SCALE PIGMENTATION

In reports (9, 10, 11) dealing with the parasitism of the smudge organism it has been shown that the host tissues contain two classes of toxic substances. One of these was found in the cell sap, the other was associated with pigmentation in the dried outer scales. It was found that water extracts from the outer dry colored scales contained toxic substances which, in sufficient concentration, either entirely prevented spore germination or caused abnormal germ-tube development. As the concentration of the extract was reduced, the toxic effects were proportionally decreased until eventually normal germination and growth occurred. Water extracts from the outer dried white scales did not inhibit spore germination or germ-tube development. It was concluded, therefore, that the resistance of colored onions to the smudge disease was due to a substance or substances either closely associated or identical with the red and yellow pigments present in the outer scales. It was suggested that these water-soluble toxins of the host were located in an advantageous position to serve as a barrier to the invasion of the parasite. In nature these toxic substances might readily dissolve into the moisture which comes in contact with the pigmented scales, producing a natural fungicidal effect on the parasite. It has been demonstrated (6), that pigmented scales which had been badly bleached by the action of meteoric or soil water contain less toxin than scales in which pigment preservation is at a maximum. In recent studies (5) a toxic entity, protocatechuic acid, was isolated from colored scales which was not present in white scales. This phenolic acid was found in a free state in colored outer scales. In dilutions of 1 to about 3,000 its toxicity resembled that of the crude water extract from which it was isolated.

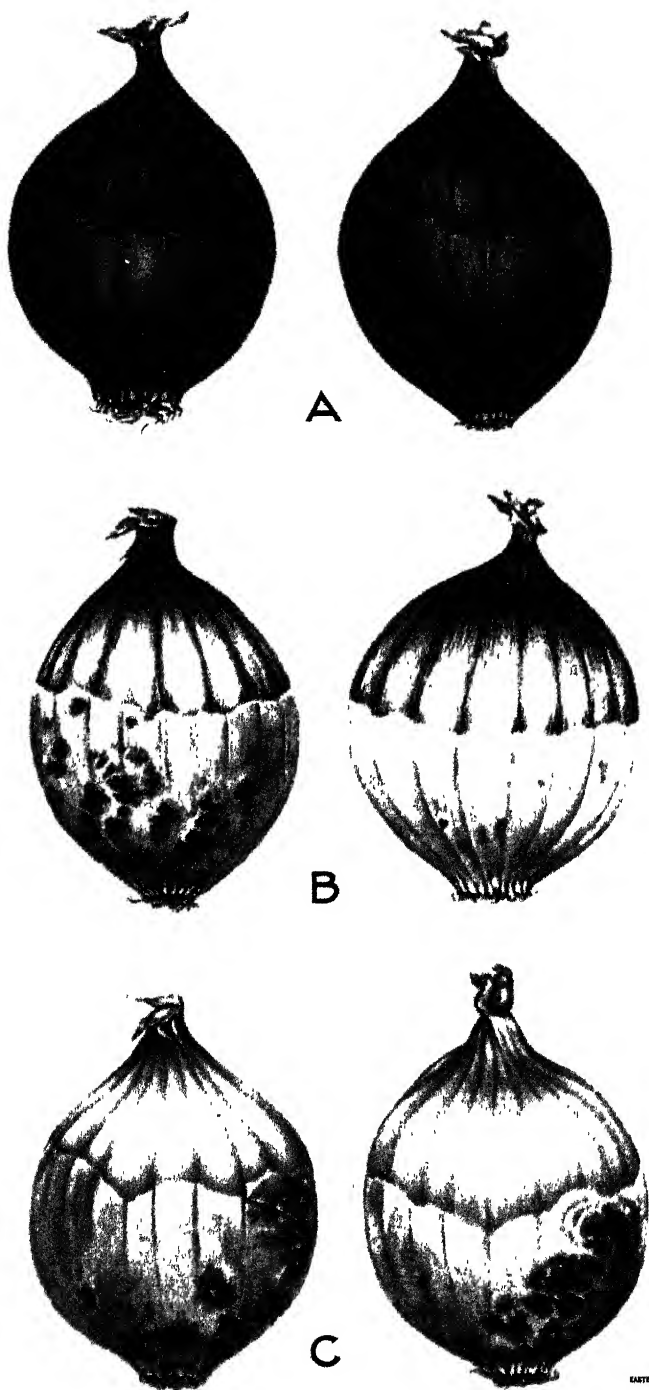
NATURE OF THE MATERIAL AND EXPERIMENTAL  
PROCEDURE

Although varieties of common onions vary somewhat in intensity of color, they can be roughly grouped into red, yellow, and white classes. Many varieties, like St. Marie Rose and Australian Brown, lie on the border line between three groups. The pigments of onion scales are solutes in the cell sap of the outer epidermal layer. A dilute color first appears when the plants are about half grown. The intensity increases during development and at maturity pigmentation is at a maximum in the dried outer scales. Epidermal



Typical susceptible white onion bulbs infected with smudge are pictured at the top (A). Note the black smudged appearance of the bulb on the right and the characteristic concentric circles of mycelium on the bulb on the left. Resistant red (B) and yellow (C) bulbs are shown below





Typical red bulbs (A), red neck bulbs (B), and white bulbs (C), after the dried outer scales have been removed from the upper portions. Note the splashes of red on the fleshy scales of red neck bulbs (B) in contrast to the greenish white appearance of the fleshy scales of the white bulbs (C). Onion smudge appears on three of the lower four bulbs





cells of yellow varieties turn a deep brownish-yellow when treated with alkalis, a reaction typical of flavones (?). Epidermal cells of red varieties turn pink in acid and green in alkaline solutions, which are characteristic reactions of anthocyanins.

In nature the common onion is a highly cross-pollinated plant. Self-pollination usually results in a loss of general plant vigor and in forms of sterility, the nature of which has not been determined. A few fertile inbred lines have been obtained which exhibited a fair amount of vigor after three generations of self-pollination. Considerable embryo degeneration occurred in both cross-pollinated and self-pollinated lines.

Crosses were made in the field and in the greenhouse. The umbels were allowed to develop normally until a few flowers opened and then the opened and the small immature flowers were plucked. The inflorescences were washed with water before the flowers were emasculated. Finely pointed tweezers were used to clip off the top portions of the perianth segments and to remove the anthers. From 40 to 100 flowers were worked on each umbel. Cross-pollinations were effected by rubbing dehiscing anthers on the stigmas of emasculated flowers. Self-pollinations were made by covering the umbels before the flowers opened. All pollinating was carefully controlled by covering the inflorescences with manila or vegetable parchment bags.

Since segregation was observed in the  $F_1$  progenies of reciprocal crosses between red and white onions, tests were made to determine whether or not apogamous development had occurred. From 10 to 15 flowers in 17 different umbels were emasculated in the usual manner, but no pollen was applied to the stigmas. Some of these unfertilized ovaries showed parthenocarpic development up to about one-half the normal size of ripening fertilized ovaries but then degenerated. These tests also demonstrated the thoroughness of the crossing technic employed.

Approximately 50 per cent of the various crosses attempted were successful. The usefulness of many of the crosses obtained was seriously impaired because (1) many or all the plants failed to set seed in later generations, (2) seed bulbs were lost as a result of storage rots, and (3) seedling progenies were destroyed by damping-off fungi.

Sporulating cultures of *Colletotrichum circinans* grown on potato-dextrose agar were used to make up spore suspensions of the smudge organism. Bulbs growing in the field during 1924 were inoculated with the spore suspension by means of a hand-pressure spray. Conditions were favorable for the development of the disease, and with few exceptions all the white bulbs showed typical smudge lesions. With the exception of relatively inconspicuous lesions on the unpigmented neck portions of some of the colored bulbs, all the red and yellow onions appeared to be immune. Sporulating cultures were not available for inoculating the 1925 crop, so diseased scales from onions infected with smudge were macerated with sand and applied along with mascerated mycelial cultures. Weather conditions were unfavorable for the development of the disease; consequently resistance records could not be obtained. Two field inoculations of mixtures of spore suspension and macerated mycelium from pure cultures were made during the summer of 1926. Warm weather and frequent rains following the inoculations favored disease development. Typical smudge lesions were prevalent at harvest time. A fairly high

percentage of disease-free white bulbs were observed. During 1927 two spore-suspension inoculations were made in the field. Smudge symptoms developed rapidly, and at harvest time many bulbs were badly diseased. Although care was taken to distribute the inoculum evenly, and two applications at intervals of two weeks were made to insure uniform infestation, an uneven distribution of the disease on white bulbs was noted at harvest time. In general the plants which developed the major portion of the bulb above the soil line were less diseased than those which had grown deeper in the soil. Low and poorly drained areas of the plot contained a larger percentage of infected white bulbs than well-drained areas. These results indicated that the methods of field inoculation employed offered a very crude measure of disease resistance.

Tests employed earlier by another investigator (10) were used to demonstrate variation in the amount of toxic materials in the outer scales of the white bulbs of  $F_2$  and  $F_3$  progenies resulting from reciprocal crosses of red and white onions. The method used was as follows: Drops of distilled water containing spores of the fungus were placed on clean glass slides, two drops on each slide, and disks of onion scales about 6 millimeters in diameter, made with a paper punch, were placed in the drops, usually two such pieces in each drop. The slides were then put in Petri dishes lined with moistened filter paper which served as moist chambers. Observations on the amount and character of spore germination were made and recorded after 18 to 48 hours.

Probable errors and goodness of fit were determined wherever necessary. The probable errors of Mendelian ratios were calculated according to the formula  $0.6745 \sqrt{p \times q \times n}$ , where  $n$  is the total number of individuals in the population,  $p$  and  $q$  are the two elements in a ratio, as 0.75 and 0.25, 0.5 and 0.5, 0.81 and 0.19, whose sums total 1.000. The probable errors thus obtained were compared with the deviations of the actual numbers from those expected on the basis of the given ratio. For tests of goodness of fit in cases where the number of classes was more than two, use was made of the  $\chi^2$  method as suggested by Harris (1).

#### WORKING HYPOTHESES FOR COLOR INHERITANCE

Five different genes are necessary to account for the genetic behavior of the four bulb color classes—red, yellow, red neck, and white—observed in the onion stocks dealt with in this paper. (Pls. 1 and 2.) These five genes are given the following symbols:

- (1)  $I$ —a gene for incomplete inhibition of color.
- (2)  $i$ —a gene allowing expression of color.
- (3)  $W$ —a gene for red pigment.
- (4)  $W^y$ —a gene for yellow pigment.
- (5)  $w$ —a gene for white.

The genes  $W$ ,  $W^y$ , and  $w$  are considered multiple allelomorphs showing independent inheritance of the genes  $I$  and  $i$ , which constitute a factor pair. The members of the allelomorphic series and the independent factor pair exhibit the following relations:

- $I$  is incompletely dominant to  $i$ .
- $W$  is dominant to  $w$ .
- $W$  is dominant to  $W^y$ .

The relationship between the gene  $W^v$  and the gene  $w$  is not clearly understood.

The phenotypic formulae assigned to the four bulb color classes under consideration are as follows:

- (1)  $Wi$ —red.
- (2)  $W^v i$ —yellow.
- (3)  $W I i$ —red neck.
- (4)  $W I I$   
 $w I$   
 $w i$  }—white.

From an examination of the factorial assignments listed above it is evident that the various bulb color classes include the following genotypes: Red,  $W W i i$ ,  $W w i i$ , and  $W W^v i i$ ; yellow,  $W^v W^v i i$  and  $W^v w i i$ ; red neck,  $W W I i$  and  $W w I i$ ; white,  $W W I I$ ,  $W w I I$ ,  $w w I I$ ,  $w w I i$ , and  $w w i i$ .

#### EXPERIMENTAL REPORT

All the material pertaining to the genetic relations of red and white classes is considered first. This includes the red-neck class found among genetically white plants. Next, the genetic relations of the red, yellow, and white classes are considered. Applicable data from all four color classes are then used to indicate the relation of the color factors to disease resistance.

#### RED AND WHITE COLOR RELATIONS

Reciprocal crosses were made between red and white open-pollinated stocks of onions. The red parent selfed resulted in 14 red bulbs, showing that it was probably homozygous for factors controlling the production of red pigment. Attempts to self the white parent failed. Red and white bulbs appeared in the  $F_1$  of the red and white crosses. (Table 1.) This at once indicated that the white parent was probably heterozygous for factors controlling pigment production.

Attempts were made to self all the  $F_1$  plants, but because of poor bulb development and storage rots,  $F_2$  and  $F_3$  progenies were obtained from only six  $F_1$  individuals. Fortunately, of the six, three were white and three were red. Each of the three  $F_1$  white bulbs was also crossed with a different  $F_1$  red bulb.

The  $F_1$ ,  $F_2$ , and  $F_3$  data from reciprocal crosses No. 2 and No. 3 indicate that two factor pairs control the expression and production of red pigment. The  $I i$  factor pair involving inhibition of color was found to be heterozygous in the open-pollinated  $P_1$  white bulb. The other factor pair governing the production of red pigment proved to be homozygous dominant in the  $P_1$  red bulb and homozygous recessive in the  $P_1$  white bulb.

The parental genotypic formulae involved may be given as follows:

- Cross No. 2 (Red×White),  $W W i i \times w w I i$ .  
 Cross No. 3 (White×Red),  $w w I i \times W W i i$ .

Under such circumstances the red  $F_1$  bulbs should have the genetic formula  $W w i i$  and the white  $F_1$  bulbs should have the genetic formula  $W w I i$ , and they should occur in equal numbers.

TABLE 1.— $F_1$  data from reciprocal crosses between red and white onion bulbs

Cross No.	Pedigree numbers	Parental genotypes	F <sub>1</sub> progenies		Deviation	Dev. P. E.
			Red	White		
2.....	R <sub>11</sub> ×W <sub>22</sub> .....	WWII×wwII.....	9	7	1.0±1.4	0.7
3.....	W <sub>22</sub> ×R <sub>11</sub> .....	wwII×WWII.....	9	3	3.0±1.2	2.5
Total.....			18	10	4.0±1.8	2.2
Theoretical, 1:1.....			14	14		

When the three remaining white  $F_1$  plants were self-fertilized they exhibited distinct 3:13 ratios of red and white bulbs. The three red  $F_1$  plants yielded 3:1 ratios of red and white bulbs. The results of these  $F_2$  progenies are presented in Table 2.

TABLE 2.— $F_2$  data from reciprocal crosses between red and white onion bulbs

F <sub>1</sub> bulb No.	F <sub>1</sub> genotypic formulae	F <sub>2</sub> progenies		Deviation	Dev. P. E.	F <sub>1</sub> bulb No.	F <sub>1</sub> genotypic formulae	F <sub>2</sub> progenies		Deviation	Dev. P. E.
		Red	White					Red	White		
2-1.....	WwII.....	31	137	0.5±3.4	0.1	2-4.....	Wwii.....	50	20	2.5±2.4	1.0
2-2.....	WwII.....	28	143	4.1±3.4	1.2	2-5.....	Wwii.....	67	38	11.8±3.0	3.9
3-1.....	WwII.....	32	166	5.1±3.7	1.4	3-2.....	Wwii.....	15	4	.8±1.3	.6
Total.....		91	446	9.7±6.1	1.6	Total.....		132	62	13.5±4.1	3.3
Theoretical, 3:13.....		100.7	436.3			Theoretical, 3:1.....		145.5	48.5		

A further test of the validity of the hypothesis offered is afforded by the results of progeny tests in generations beyond the  $F_2$ . Nine  $F_2$  genotypes showing the breeding behavior indicated are called for, as follows:

- 1 WWII—all white.
- 2 WwII—all white.
- 2 WWIi—1 red : 3 white.
- 4 WwIi—3 red : 1 white.
- 1 wwII—all white.
- 2 wwIi—all white.
- 1 WWii—all red.
- 2 Wwii—3 red : 1 white.
- 1 wwii—all white.

A total of 105 white  $F_2$  plants were selfed and progeny tests were conducted on a relatively large scale. Progenies from selfed white  $F_2$  plants are arranged in Table 3.

TABLE 3.— $F_3$  data from selfed white  $F_2$  plants of onion progenies segregating for red and white in 3:13 ratios

Parent bulbs having genotypic formulae $WwIi$ or $WWIi$						Parent bulbs having genotypic formulae $WWII$ , $WwII$ , $wwII$ , $wwIi$		
$F_2$ bulb No.	$F_3$ progenies		Ratio for 24 individuals		Deviation in red class		$F_2$ bulb No.	All white $F_3$ progenies
	Red	White	Red	White	-	+		
46-2	8	44	3 7	20 3	1.3		48-1	1
46-4 <sup>a</sup>	3	7					48-2	81
47-1	2	16	2 7	21 3	2.3		48-4	20
47-3	14	19	10 2	13 8		5 2	49-7	47
47-4 <sup>a</sup>	1	9					52-1	374
47-5	4	24	3 4	20 6	1.6		53-5 <sup>a</sup>	11
49-2	10	24	7 0	17 0		2 0	54-3	326
51-1	79	323	4 7	19 3	.3		54-7	94
51-3	2	14	3 0	21 0	2.0		54-9	39
51-4	6	25	4 6	19 4	.4		54-10	139
52-2	12	57	4 2	19 8	.8		54-12	44
52-3 <sup>a</sup>	1	6					54-13	49
52-4	11	33	6 0	18 0		1 0	54-14 <sup>a</sup>	14
53-2	11	26	7 1	16 9		2 1	54-15	107
53-4	4	27	3 1	20 9	1.9		54-16	48
53-7	3	19	3 3	20 7	1.7		55-1 <sup>a</sup>	13
53-8	2	17	2 5	21 5	2.5		55-2 <sup>a</sup>	3
53-9	9	30	5 5	18 5		.5	55-4 <sup>a</sup>	9
53-10	142	626	4 4	19 6	.6		55-6	49
54-4	23	80	5 4	18 6		.4	55-8	26
54-17	3	16	3 8	20 2	1.2		55-9	231
54-18	4	33	2 6	21 4	2.4		55-11 <sup>a</sup>	8
54-19	22	49	7 4	16 6		2 4	55-12	20
54-20 <sup>a</sup>	1	2					55-13	504
55-5	17	39	7 3	16 7		2 3	55-18	132
55-10	7	49	3 0	21 0	2 0		55-19	129
55-15	34	155	4 3	19 7	.7		55-20	73
55-17 <sup>a</sup>	3	12					55-22	30
11b-1 <sup>a</sup>	6	7					55-23	107
11c-1	96	334	5 4	18 6		.4	55-26	184
57-4	71	218	5 9	18 1		.9	55-27	29
57-2 <sup>a</sup>	1	5					55-28 <sup>a</sup>	11
57-5	12	120	2 2	21 8	2 8		55-30	105
58-1	45	352	2 7	21 3	2 3		55-33	21
58-2	14	48	5 4	18 6		.4	59-34	20
58-3	5	31	3 3	20 7	1 7		59-1	171
58-4	4	26	3 2	20 8	1 8		59-2 <sup>a</sup>	11
58-55	4	36	2 4	21 6	2 6		59-5	46
58-7	11	20	8 5	15 5		3 5	59-7	90
59-1	57	217	5 0	19 0	0	0	59-12	25
59-3	22	91	4 7	19 3	.3		59-14	78
59-4	12	106	2 4	21 6	2 6		59-16	28
59-6	18	139	2 8	21 2	2 2		59-19	32
59-11	20	113	3 6	20 4	1 4		59-20 <sup>a</sup>	4
59-13	14	85	3 4	20 6	1 6		60a-2	40
60a-1	3	36	1 8	22 2	3 2		60a-6	43
60a-16	62	302	4 1	19 9	.9		60a-7	24
60a-21a	1	9					60a-14	22
60b-1	4	16	4 8	19 2	.2		60a-20	83
60b-3	22	92	4 6	19 4	.4			
60b-5	10	29	6 2	17 8		1 2		
60b-7	23	154	3 1	20 9	1 9			
60b-8	4	25	3 3	20 7	1 7			
60b-9	7	58	2 6	21 4	2 4			
60b-12	35	130	5 1	18 9		.1		
60b-15	4	18	4 4	19 6	.6			
Total	1,008	4,541						3,727
Total number of progenies						33	14	
Theoretical, 2:1						31.3	15.7	
Deviation						1.7±1.9		
Dev.						1.1		
P. E.								
Total number of progenies						48	40	
Theoretical, 6:7						40.6	47.4	
Deviation						7.4±3.2		
Dev.						2.3		
P. E.								

<sup>a</sup> Progenies containing less than 16 individuals are not considered because of the uncertainty in distinguishing true breeding from segregating families.

The  $F_3$  data from selfed white  $F_2$  plants of progenies segregating for red and white in 3:13 ratios show that the total number of segregating families and true-breeding white families agrees with the expected 6:7 ratio. With the number of individuals available it is not possible to differentiate definitely between the two types of ratios, 1:3 and 3:13, expected among the segregating progenies because their numerical proportions are quite similar. Critical progeny tests of some of the segregating  $F_3$  families will be presented later. At this point it is of interest to know whether the ratios obtained fall into the expected narrow range. Therefore, the two types of ratios were combined in the proportion in which they would be expected to occur. Thus, the 1:3 ratio was doubled and added to the 3:13 ratio, resulting in a ratio of 5:19. This combined ratio allows for the occurrence of twice as many plants having the genetic formula  $WwIi$  as those having the genetic formula  $WWIi$ . In order to offset to a certain degree the influence of variation in numbers of individuals within families which range between 16 and 768, the actual ratio was weighted in terms of 24 individuals. Examination of the individual entries in Table 3 shows that, in general, the deviations are not excessively large. On the basis of chance the plus and minus deviations for the 48 families should approximate a 2:1 ratio. The 33 minus deviations and the 14 plus deviations found in the red class are in accord with the hypothesis presented in this paper.

#### PHENOTYPIC EXPRESSIONS OF THE INTERACTION OF THE COLOR FACTORS

It is of interest to demonstrate that 1:3 and 3:13 ratios of red and white are present in the segregating  $F_3$  progenies. One line of evidence is presented by the interaction of the  $Ww$  factor pair with the  $Ii$  factor pair.

Cream-colored bulbs of varying intensity have been observed in fairly large numbers in all progenies possessing the  $W$  gene and segregating for the  $I$  gene. Only an occasional light cream-colored bulb has been noted in progenies breeding true for white. Another condition, called red neck, has also been observed frequently on genetically white bulbs in most of the progenies carrying the  $W$  gene and segregating for the  $I$  gene. These observations indicate that the two conditions, cream and red neck, are similar in nature and that they are the phenotypic expression of the interaction of the heterozygous  $Ii$  factor pair and the gene  $W$ . Red neck usually appears as narrow, red areas on the surface of the inner fleshy scales in the neck region of the bulbs. (Pl. 2.) It varies in intensity from almost full red to minute red areas that can be seen only with the aid of a hand lens. In nearly all cases bulbs having red neck were cream colored, though many cream-colored bulbs do not show red neck.

A classification of the segregating and the true white-breeding  $F_3$  progenies shows that in only 1 case out of 40 were true-breeding white  $F_2$  bulbs classified as cream. Fifteen segregating  $F_2$  bulbs out of 46 were classified as white. These relations clearly show that the cream character is closely associated with the heterozygous condition of the  $Ii$  factor pair in the presence of the  $W$  gene.

Since the cream character was difficult to classify and a close correlation was noted between it and the red-neck character it was considered advisable to classify the  $F_3$  bulbs according to the presence of red color on the inner fleshy scales. Obviously the expression of a

delicate color character protected by plant tissues should be more constant than one expressed in external plant tissues which are exposed to weathering conditions. Then, too, the outer scale of the bulb is often lost in harvesting, which might cause a cream bulb to appear white.

The available data on the occurrence of these intermediate characters associated with the heterozygous condition of the *I* gene in the presence of the *W* gene are presented in Table 4. Many of the bulbs could not be classified because of field and storage rots or poor bulb development.

TABLE 4.—Occurrence of white and cream and red-neck colored  $F_3$  onion bulbs

F <sub>2</sub> bulb No.	F <sub>3</sub> progenies segregating for genetically red and white bulbs		F <sub>2</sub> bulb No.	F <sub>3</sub> progenies breeding true for genetically white bulbs	
	White and cream	Red neck		Pure white	White and cream
46-2	26	—	48-1	10	—
46-4	2	5	48-2	33	—
47-1	11	—	48-4	17	—
47-3	11	8	52-1	76	—
47-5	22	1	54-3	—	123
49-2	8	—	54-7	—	94
51-4	4	—	54-10	139	—
52-2	18	25	54-12	35	—
52-4	11	0	54-13	—	9
53-2	11	7	54-15	—	107
53-7	16	—	55-6	—	13
53-8	11	6	55-13	—	498
53-10	247	284	55-18	106	—
54-4 <sup>a</sup>	62	—	55-19	97	—
54-19 <sup>b</sup>	40	—	55-20	—	17
55-5	8	1	55-23	—	103
55-10	17	—	55-26	37	—
55-15	49	—	55-27	9	—
55-17	3	—	55-30	63	—
57-4	72	113	55-34	23	—
57-5	64	53	56-1	—	33
58-1	157	93	59-5	42	—
58-2	19	20	59-7	50	—
58-3	9	14	59-12	—	16
58-4	4	5	59-19	—	21
58-5	27	6	60a-2	22	—
58-7	12	7	60a-6	—	25
59-1	148	60	60a-7	—	19
59-3	56	32	60a-14	—	11
59-4	45	39	60a-20	—	74
59-6	85	48			
59-11	38	20			
59-13	60	19			
60a-1	44	—			
60a-16	12	8			
60b-3	43	28			
60b-5	6	18			
60b-7	75	71			
60b-8	13	12			
60b-9	45	8			
60b-12	19	26			
60b-15	8	3			
Total number of progenies...	42	32	Total number of progenies...	15	15

<sup>a</sup> An occasional red-neck bulb was observed.

<sup>b</sup> Red-neck bulbs present.

The data in Table 4 show that the red-neck character is associated with progenies segregating for genetically red and white bulbs, indicating that its expression depends upon the heterozygous condition of the *Ii* factor pair in the presence of the *W* gene. In only



2 of the 30 families breeding true for genetically white bulbs did an occasional red-neck bulb appear. The occurrence of a few red-neck bulbs in families breeding true for genetically white bulbs is not surprising if this character is dependent upon a heterozygous condition. The expression of color in full red bulbs does not take place until the plants are about half grown, showing that color differentiation is dependent upon physiological changes occurring late in the development of the plant. Likewise the red-neck character is most strongly expressed in well-matured bulbs.

Table 5 shows that in the  $F_3$  progeny No. 262, segregating for red and white, all the genetically white, red-neck bulbs carried the  $W$  gene and were heterozygous for the  $I$  gene and all the pure white bulbs lacking the red-neck character possessed one of the five possible genotypes as follows:  $WWII$ ,  $WwII$ ,  $wwII$ ,  $wWIi$ , and  $wwii$ .

TABLE 5.—Breeding behavior of pure white and red-neck  $F_3$  onion plants

F <sub>3</sub> bulb No.	F <sub>3</sub> phenotype	F <sub>4</sub> progenies		F <sub>3</sub> bulb No.	F <sub>3</sub> phenotype	F <sub>4</sub> progenies	
		Red	White			Red	White
262W-2a.....	Pure white.....	0	20	262Rn-1a.....	Red neck.....	3	19
262W-3a.....		0	8	262Rn-1.....		3	22
262W-3.....		0	17	262Rn-2.....		5	18
262W-4a.....		0	16	262Rn-3.....		2	17
262W-5a.....		0	15	262Rn-4.....		12	38
262W-7.....		0	14	262Rn-5.....		8	48
262W-9.....		0	8	262Rn-6.....		7	32
262W-10.....		0	23	262Rn-7.....		2	31
262W-14a.....		0	43	262Rn-8.....		8	31
262W-15a.....		0	7	262Rn-9a.....		4	13
262W-20a.....		0	108	262Rn-9.....		0	13
				262Rn-10.....		6	38
				262Rn-17.....		2	19
				262Rn-18a.....		0	4
				262Rn-19a.....		6	18
Total.....		0	279	Total.....		68	361

Attempts were made to test out the five genotypically different whites occurring in the 3 red : 13 white ratio. The types of progenies resulting from crosses between the five different whites and a true-breeding red should be as follows:

P <sub>1</sub> genotypes	F <sub>1</sub> phenotypes
1 $WWII \times WWii$ .....	All white.
2 $WwII \times WWii$ .....	Do.
1 $wwII \times WWii$ .....	Do.
2 $wWIi \times WWii$ .....	1 white : 1 red.
1 $wwii \times WWii$ .....	All red.

Ten crosses between true-breeding white and red plants are presented in Table 6. Red plants from an all red  $F_3$  progeny of 138 individuals resulting from a selfed red  $F_2$  plant were used for the red parents. Six different white  $F_2$  plants which gave all white  $F_3$  progenies were used for the white parents. All but one of the red parents used in the crosses were selfed and found to be homozygous red.

TABLE 6.— $F_1$  data from crosses between true-breeding white and red onion plants

Cross No.	Pedigree Nos.	$F_1$ progenies		Cross No.	Pedigree Nos.	$F_1$ progenies	
		Red	White			Red	White
45.....	54-12×181-11.....	0	16	52.....	181-3×60a-14.....	0	1
46.....	181-11×54-12.....	0	17	53.....	181-16×60a-14.....	3	4
47.....	181-7×55-18.....	0	27	54.....	181-18×60a-14.....	1	0
48.....	55-18×181-6.....	0	19				
49.....	55-19×181-11.....	0	12				
50.....	55-20×181-11.....	0	4				
51.....	181-15×55-30.....	0	23				
Total.....		0	118	Total.....		4	5
Expected.....		0	118	Expected, 1:1.....		4.5	4.5

Of the six different whites used in the crosses five gave all white plants and one gave red and white plants in the ratio of 1:1. On the assumed theoretical basis there are four chances in seven of obtaining white plants that would give all white offspring when crossed with homozygous red plants and two chances in seven of obtaining white plants that would give a ratio of one red to one white when crossed with homozygous red plants. The results of the crosses listed in Table 6 agree very closely with the expected. Five out of six white plants used in crosses with red plants gave all white offspring and only one out of six gave a 1:1 ratio of red and white. These results indicate the presence of white plants with the genotypic formula  $wwIi$  in progenies segregating for 3:13 ratios of red and white. They also give some evidence of the occurrence of the three expected genotypes  $WWII$ ,  $WwII$ , and  $wwII$ .

The distribution of the red-neck character indicates strongly that genetically white plants having the genotypic formulae  $WWIi$  or  $WwIi$  are present in progenies segregating for red and white. It has been shown in Table 5 that red-neck bulbs are heterozygous for the  $Ii$  factor pair. Therefore, in a consideration of only the white and red-neck classes, a selfed white plant having the genotypic formula  $WwIi$  should give six red-neck plants to seven white or cream plants, and a selfed white plant having the genotypic formula  $WWIi$  should give two red-neck plants to one white plant. Even though the expression of the heterozygous character red neck is not constant, the numerical difference between a 6:7 and a 2:1 ratio should be sufficiently large to enable one to distinguish between these two groups.

If the segregating progenies having a larger number in the red-neck class than in the white and cream class are grouped (Table 7) it becomes apparent that a grouping of certain 1:3 ratios has been effected. The 1 red:3 white ratio fits the total population better than the 3 red:13 white ratio. The two progenies 57-4 and 60b-5, which show fairly close agreement with a 2:1 ratio of cream red-neck bulbs to white and cream bulbs (Table 4), also show a close fit to the 1:3 ratio of red and white ( $\frac{\text{Dev.}}{\text{P. E.}} = 0.3$  and 0.2, respectively) (Table 3). Furthermore, progeny tests of these two families, which are recorded in Table 8, show that the red individuals were all homozygous, as would be expected in the 1 red:3 white ratios.

TABLE 7.—*F*<sub>3</sub> onion progenies segregating for red and white having a larger number in the red-neck class than in the white and cream class

F <sub>2</sub> bulb No.	F <sub>3</sub> progenies		1:3 ratio		3:13 ratio		F <sub>3</sub> white class	
	Red	White	Deviation	Dev. P. E.	Deviation	Dev. P. E.	White and cream	Red neck
46-4.....	3	7	0.5	—	1.1	—	2	5
52-2.....	12	57	5.2±2.4	2.2	9±2.2	0.4	18	25
57-4 <sup>a</sup> .....	71	218	1.3±5.0	3	16.8±1.5	3.7	72	113
58-2 <sup>a</sup> .....	14	48	1.5±2.3	.7	2.4±2.1	1.1	19	20
58-3.....	5	31	4.0±1.8	2.2	1.8±1.6	1.1	9	14
58-4.....	4	26	3.5±1.6	2.2	1.6±1.4	1.1	4	5
60b-5 <sup>a</sup> .....	10	29	.3±1.8	2	2.7±1.6	1.7	6	18
60b-12.....	35	130	6.3±3.8	1.7	4.1±3.4	1.2	19	26
11c-1.....	96	334	11.5±6.1	1.9	15.4±5.5	2.8	145	160
Total.....	250	880	32.5±9.8	3.3	38.1±8.8	4.3	—	—

<sup>a</sup> Progeny tests demonstrate that they are 1:3 ratios.

Further evidence in support of the assumption that the segregating *F*<sub>3</sub> progenies are either 1 : 3 or 3 : 13 ratios of red and white is offered by the *F*<sub>3</sub> progeny tests of the red classes. All the red individuals in the 1 : 3 ratio should have the genotypic formula *WWii*, while those in the 3 : 13 ratio should have the genotypic formulae *Wwii* and *WWii* in the proportion of 2 : 1. This would yield homozygous red progenies from 1 : 3 ratios and 2 heterozygous red and white progenies to 1 homozygous red progeny from the 3 : 13 ratios. The differentiation also furnishes additional proof that white plants having the genotypic formulae *WWIi* and *WwIi* are present in progenies segregating for red and white in 3 : 13 ratios, since upon self-pollination, white plants with the genetic constitution *WWIi* and *WwIi* should yield the 1 : 3 and 3 : 13 ratios, respectively. The breeding behavior of red individuals from 17 segregating *F*<sub>3</sub> progenies is presented in Table 8. Of the 17 progenies tested, 6 contained only homozygous red individuals and 11 contained both homozygous and heterozygous red plants. In this case there is a close agreement between the observed and expected ratios, since twice as many *WwIi* genotypes as *WWIi* genotypes are expected in a 3 : 13 ratio.

All of the *F*<sub>4</sub> progenies which contained less than 12 individuals were not included in the tables because of the uncertainty in classifying the true-breeding red families. The odds against a true-breeding progeny of 12 red individuals being a segregating progeny of 3 red : 1 white are about 22 to 1.

TABLE 8.— $F_4$  data from self-pollinated red  $F_3$  onion plants of progenies segregating for red and white in 3:13 and 1:3 ratios

F <sub>2</sub> bulb No.	F <sub>3</sub> bulb No.	Progenies from 3 : 13 ratios				F <sub>2</sub> bulb No.	F <sub>3</sub> bulb No	Progenies from 1 : 3 ratios	
		Segregating F <sub>4</sub> progenies		Nonsegregating F <sub>4</sub> progenies				F <sub>4</sub> progenies	
		Red	White	Red	White			Red	White
47-5	173R-1			30	0	47-3	170R-1	45	0
	173R-2			16	0		170R-2	29	0
	173R-3	29	14				170R-3	20	0
	173R-4	11	2				170R-4	56	0
49-2	177R-2			70	0	52-4	170R-5	21	0
	177R-3	39	13				170R-8	24	0
	177R-4	36	17				170R-9	26	0
	177R-5	45	14				170R-10	36	0
51-1	177R-6			56	0	53-10	170R-11	33	0
	188R-1			53	0		193R-1a	32	0
	188R-2			40	0		193R-1b	95	0
	188R-3	56	21				193R-2	15	0
55-5	188R-4	48	16			57-4	193R-6	36	0
	188R-6	26	14				200R-1	57	0
	188R-7	11	5				200R-3	55	0
	188R-8			20	0		200R-6	43	0
55-15	188R-9	33	12			58-2	200R-7	67	0
	188R-10	61	27				200R-8	79	0
	214R-2	21	6				200R-9	42	0
	214R-4	20	5				200R-10	32	0
57-5	214R-5	36	6			60h-5	234R-1	47	0
	214R-8			14	0		234R-2	131	0
	214R-11a	49	15				234R-23	73	0
	214R-12			39	0		234R-25	13	0
58-1	214R-14	20	6			60h-5	234R-26	129	0
	214R-15a			18	0		234R-28	122	0
	222R-1	9	3				234R-29	158	0
	222R-5			48	0		234R-36	40	0
59-1	222R-6	31	8			60h-5	234R-37	111	0
	222R-8	22	6				234R-38	92	0
	235R-1	32	16				234R-40	93	0
	235R-2	17	6				234R-41	105	0
59-3	235R-3	50	15			60h-5	234R-42	111	0
	235R-4			36	0		237R-1	42	0
	235R-5	48	18				237R-2	47	0
	235R-6	54	20				237R-3	23	0
59-5	235R-7	11	8			60h-5	237R-4	27	0
	235R-8	65	22				237R-5	16	0
	235R-9			21	0		237R-6	13	0
	235R-10			19	0		237R-7	20	0
59-7	236R-1	27	9			60h-5	237R-8	65	0
	236R-2	20	7				237R-10	18	0
	236R-3			41	0		263R-1	20	0
	236R-4	88	30				263R-2	12	0
59-9	236R-6			57	0	60h-5	263R-3	35	0
	236R-7	46	16				263R-4	37	0
	236R-9	30	10						
	236R-12	43	13						
59-11	236R-13			32	0	60h-5			
	236R-23	22	5						
	241R-1	77	22						
	241R-2	64	21						
59-13	241R-3	91	28			60h-5			
	241R-4			70	0				
	241R-6	54	12						
	241R-14	60	17						
59-15	241R-16	30	10			60h-5			
	241R-19	50	8						
	241R-21	50	19						
	241R-24	43	19						
59-17	241R-25	48	15			60h-5			
	241R-27			81	0				
	243R-2	31	9						
	243R-3	100	35						
59-19	243R-5	16	7			60h-5			
	243R-6	55	21						
	243R-7	88	32						
	243R-8	61	22						
59-21	243R-9			67	0	60h-5			
	243R-10	77	25						
	243R-11	108	35						

TABLE 7.—*F<sub>3</sub> onion progenies segregating for red and white having a larger number in the red-neck class than in the white and cream class—Continued*

F <sub>2</sub> bulb No.	F <sub>3</sub> bulb No.	Progenies from 3 : 13 ratios				F <sub>2</sub> bulb No.	F <sub>2</sub> bulb No.	Progenies from 1 : 3 ratios	
		Segregating F <sub>1</sub> progenies		Nonsegregating F <sub>1</sub> progenies				F <sub>1</sub> progenies	
		Red	White	Red	White			Red	White
9-3	243R-12	53	22						
	243R-14			129	0				
	243R-15			68	0				
	249R-1			16	0				
	249R-2	37	14						
	249R-4	61	18						
59-13	249R-5	20	3						
	249R-6	11	3						
	249R-7	19	8						
	249R-8			31	0				
	249R-9			22	0				
	249R-10	24	16						
60a-16	249R-11	53	22						
	249R-12	41	24						
	249R-14	25	13						
	258R-1	38	13						
	258R-3			17	0				
	258R-4			78	0				
	258R-5	65	19						
	258R-6			74	0				
Total		2,706	937	1,263	0			2,443	0
Theoretical, 3 : 1		2,732.3	910.7						
Deviation		26.3	±17.6						
Dev.		1.5							
P. E.									
Total number of F <sub>1</sub> progenies		63		28				46	
Theoretical, 2 : 1		60.7		30.3					
Deviation		2.3	±3.0						
Dev.		.7							
P. E.									
Total number of F <sub>2</sub> individuals tested				11			6		
Theoretical, 2 : 1				11.3			5.7		
Deviation				.3	±1.3				
Dev.				.2					
P. E.									

\* Small white sets are not considered, since they might have produced color upon further development.

It is of interest to note the character of the two types of segregating F<sub>3</sub> progenies which have been differentiated by means of the F<sub>3</sub> progeny tests. The data recorded in Table 9 indicate that the F<sub>3</sub> distributions agree with their F<sub>4</sub> behavior. It is true that in the case of the three progenies 53-10, 57-5, and 58-1 the fit of observation to hypothesis is not particularly close, but the progeny tests show that the red individuals include the expected genotypes in the proper proportions.

Self-pollinated red plants from progenies segregating in a 3:13 ratio are recorded in Table 10. Among the 12 red F<sub>2</sub> plants tested 7 were heterozygous and 5 were homozygous for the *Ww* factor pair. This agrees very well with the theoretical expectancy, which is a 2:1 ratio of heterozygotes to homozygotes. These data present additional evidence that the two types of expected reds occur in 3:13 ratios.

TABLE 9.— $F_3$  data from selfed white  $F_2$  onion plants of progenies segregating for red and white in 3:15 ratios

F <sub>2</sub> bulb No.	F <sub>2</sub> genotypic formulae	F <sub>3</sub> progenies		1:3 ratio		F <sub>2</sub> bulb No.	F <sub>2</sub> genotypic formulae	F <sub>3</sub> progenies		1:3 ratio	
		Red	White	Deviation	Dev. P. E.			Red	White	Deviation	Dev. P. E.
47-5.....	} <i>WWII</i>	4	24	1.3± 1.4	0.9	47-3.....	} <i>WWII</i>	14	19	5.7± 1.7	3.3
49-2.....		10	24	3.6± 1.5	2.4	52-4.....		11	33	.0± 1.9	0
51-1.....		79	323	3.6± 5.3	7.7	53-10.....		142	626	50.0± 8.1	6.2
55-5.....		17	39	6.5± 2.0	3.3	57-4.....		71	218	1.3± 5.0	.3
55-15.....		34	155	1.4± 3.6	4.4	58-2.....		14	48	1.5± 2.3	.7
57-5.....		12	120	12.8± 3.0	4.3	60b-5.....		10	29	.3± 1.8	.2
58-1.....		45	352	29.4± 5.3	5.5						
59-1.....		57	217	5.6± 4.4	1.3						
59-3.....		22	91	.8± 2.8	.3						
59-13.....		14	85	4.6± 2.6	1.8						
60a-16.....		62	302	6.3± 5.0	1.3						
Total.....		356	1,732	35.5±12.1	2.9	Total.....		262	973	46.7±10.2	4.6

Progeny No. 50b-1 is considered a homozygous red family on the basis of its progeny tests. Fourteen red individuals were tested and found to breed true for red. Five of the 15 whites were self-pollinated. Four segregated for red and white, giving a total of 16 red and 74 white individuals, and 1 segregated for white and yellow, giving 10 white and 2 yellow bulbs, indicating contamination. Only 3 yellow bulbs have appeared among approximately 10,000 bulbs from crosses Nos. 2 and 3. A mutation from red to yellow is a possible explanation. However, yellow plants were grown near plants Nos. 11-1 and 50b-1, so that crossing may have taken place.

TABLE 10.— $F_3$  data from selfed red  $F_2$  onion plants of progenies segregating for 3:15 ratios of red and white

F <sub>2</sub> bulb No.	F <sub>2</sub> genotypic formulae	F <sub>3</sub> progenies		F <sub>2</sub> bulb No.	F <sub>2</sub> genotypic formulae	F <sub>3</sub> progenies	
		Red	White			Red	White
45-1.....	} <i>W<sup>a</sup>W<sup>b</sup>ii</i>	6	2	50b-1.....	} <i>W<sup>a</sup>W<sup>b</sup>ii</i>	265	<sup>a</sup> 15
45-3.....		50	20	50-2.....		12	0
45-4.....		15	3	50-3.....		138	0
50-5 <sup>b</sup> .....		4	0	50-9.....		23	0
50-10.....		39	9	11-1.....		128	<sup>c</sup> 1
50-13 <sup>b</sup> .....		3	0				
12-7.....		6	2				
Total.....		116	36	Total.....		566	0
Theoretical, 3:1.....		114	38	Theoretical.....		566	0
Deviation.....		2.0±3.6				0	-----
Dev.....							
P.E.....		.6					

<sup>a</sup> 15 white bulbs not considered (see text).

<sup>b</sup> Progenies not considered because of possible errors in classification.

<sup>c</sup> 1 yellow bulb, which is obviously the result of contamination, since yellow has appeared only twice in progenies derived from crosses No. 2 and No. 3.

TABLE 11.— $F_3$  data from selfed red and white  $F_2$  onion plants of progenies segregating for 3:1 ratios of red and white

F <sub>2</sub> bulb No.	F <sub>2</sub> geno- typic formulae	F <sub>3</sub> progenies		Devia- tion	Dev P. E.	F <sub>2</sub> bulb No.	F <sub>2</sub> geno- typic formulae	F <sub>3</sub> progenies		Devia- tion
		Red	White					Red	White	
14c-2.....	} <i>Wwii</i> .....	8	1	1.3		13b-1.....	} <i>wwii</i> .....	0	7	0
14c-3.....		32	10	.5±1.9	0.3	14-10.....		0	30	0
62-1.....		48	10	4.5±2.2	2.0	61-1.....		0	10	0
						14c-1.....		18	0	0
						11-1.....	} <i>W'Wh</i> .....	58	0	0
Total.....		88	21	6.3±3.1	2.0	Total.....		76	47	0
Theoretical, 3:1.....		81.7	27.3							

The data presented in Table 11 demonstrate the presence of the three genotypes *WWii*, *Wwii*, and *wwii*, which would be expected if red and white plants segregating in a 3:1 ratio were self-pollinated. Of the red bulbs tested 3 are heterozygous and 2 are homozygous, which agrees with the expected 2:1 ratio. All the white bulbs behaved like double recessives.

The following crosses were made between tested  $F_1$  bulbs resulting from reciprocal crosses between red and white plants:

Cross No. 20, *WwIi* × *Wwii*.

Cross No. 21, *Wwii* × *WwIi*.

Cross No. 22, *WwIi* × *Wwii*.

Under such circumstances each of the three crosses would form 6 different genotypes in the following proportions:

1 *WWIi*.  
 1 *WWii*.  
 2 *WwIi*.  
 2 *Wwii*.  
 1 *wwIi*.  
 1 *wwii*.

The plants which appeared from these crosses are listed in Table 12. Though the numbers from the crosses are necessarily small, the actual ratios agree with the expected ratio of 3 red:5 white.

TABLE 12.—Data from crosses between  $F_1$  red and white onion bulbs

Cross No.	Pedigree No.	F <sub>1</sub> progenies		Deviation	Dev. P. E.
		Red	White		
20.....	2-2×2-5.....	8	21	2.9±1.8	1.6
21.....	2-4×2-1.....	4	23	6.1±1.7	3.6
22.....	3-1×3-2.....	3	3	0.8±0.9	.9
Total.....		15	47	8.25±2.6	3.2
Theoretical, 3:5.....		23.25	38.75		

TABLE 13.— $F_2$  data from cross No. 20

F <sub>1</sub> bulb No.	F <sub>1</sub> genotypic formulae	F <sub>2</sub> progenies		Deviation	Dev. P. E.
		Red	White		
69-2-----	} <i>Wwii</i> -----	62	26	4.0±2.7	1.5
69-3-----		31	9	1.0±1.9	.5
Total-----		93	35	3.0±2.7	1.1
Theoretical, 3:1-----		96	32		
70-1-----	<i>wwii</i> or <i>wwIi</i> -----	0	231	0	0

The  $F_2$  data presented in Table 13 show that expected heterozygous red plants *Wwii* and homozygous white plants *wwii* or *wwIi* were present in the red and white population resulting from cross No. 20.

## RED AND YELLOW COLOR RELATIONS

Two different crosses were made between red and yellow open-pollinated stocks of onions. Upon self-pollination the yellow parent that was used in both crosses gave 32 yellow bulbs, demonstrating that it was probably homozygous for factors controlling the production of yellow pigment. Self-pollinations of the two red parents  $R_{14}$  and  $R_{22}$  were not successful.

TABLE 14.— $F_1$  data from crosses between red and yellow onion plants

Cross No.	Pedigree No.	Parental genotypes	F <sub>1</sub> progenies	
			Red	Yellow
7-----	$R_{22} \times Y_8$ -----	<i>WWu</i> × <i>W<sup>y</sup>W<sup>y</sup>ii</i> -----	6	0
8-----	$Y_8 \times R_{14}$ -----	<i>W<sup>y</sup>W<sup>y</sup>ii</i> × <i>WWii</i> -----	6	0
Total-----			12	0
Theoretical-----			12	0

In every case the  $F_1$  plants were normal red. (Table 14.) This at once indicated that the gene for red *W* was dominant to the gene or genes for yellow and that the red parents were probably homozygous *WW*.

TABLE 15.— $F_2$  and back cross data resulting from crosses between red and yellow onion plants

F <sub>1</sub> bulb No.	F <sub>1</sub> genotypic formulae	F <sub>2</sub> progenies		Cross No.	Pedigree No.	Parental genotypes	F <sub>1</sub> progenies	
		Red	Yellow				Red	Yellow
7-1-----	} <i>WW<sup>y</sup>ii</i> -----	83	26	23-----	7-1 × 6-3-----	} <i>WW<sup>y</sup>ii</i> × <i>W<sup>y</sup>W<sup>y</sup>ii</i> -----	8	9
7-2-----		49	23	24-----	8-2 × 6-3-----		8	5
8-1-----		69	16	42-----	8-1 × 6-3-----		8	7
8-2-----		32	4					
Total-----		233	69	Total-----			24	21
Theoretical, 3:1-----		226.5	75.5	Theoretical, 1:1-----			22.5	22.5
Deviation-----		6.5±5.1		Deviation-----			1.5±2.3	
Dev-----		1.3		Dev-----			.7	
P. E.-----				P. E.-----				

\* One small cream-colored set included.



Only 4 of the 12 red  $F_1$  plants obtained were tested in later generations. These four were all self-pollinated and three were backcrossed to homozygous yellow plants. The progenies of the selfed  $F_1$  plants and back crosses presented in Table 15 show evidence of a simple 3:1 relationship.

The  $F_1$  and  $F_2$  data from crosses Nos. 7 and 8 indicate that a gene for yellow, designated by the symbol  $W^y$ , holds a simple Mendelian recessive relationship to the gene  $W$  for red.

The  $F_2$  red bulbs should be composed of two kinds of genotypes,  $WW^yii$  and  $WWii$ , and the  $F_2$  yellow bulbs should all have the genetic constitution  $W^yW^yii$ . The  $F_3$  progenies presented in Table 16 demonstrate that the three kinds of genotypes were present and that twice as many heterozygous as homozygous red bulbs occurred.

TABLE 16.— $F_3$  data from selfed  $F_2$  plants resulting from crosses of red and yellow onion plants

F <sub>2</sub> bulb No.	F <sub>2</sub> genotype formulae	F <sub>3</sub> progenies		F <sub>2</sub> bulb No.	F <sub>2</sub> genotype formulae	F <sub>3</sub> progenies	
		Red	Yellow			Red	Yellow
16c-2.....	} $WW^yii$ .....	2	3	65-1.....	$W^yW^yii$ .....	0	205
17a-1.....		15	8	65-3.....	do.....	0	13
64-2.....		31	16	65-4.....	do.....	0	13
68-2.....		34	8	67-3.....	do.....	0	39
68-3.....		38	4	*14c-1.....	$WWii$ .....	8	0
				64-1.....	do.....	21	0
Total.....		120	39	Total.....		29	270
Theoretical, 3:1.....		119.25	39.75				
Deviation.....		.8±3.7					
Dev.....		.2					
P. E.....							

\* One small white bulb appeared in this progeny which can not be accounted for on the basis of the assumed factorial hypothesis.

Two interesting crosses were made between white  $F_1$  plants that were assigned the genetic formula  $WwIi$  in Table 2, and red  $F_1$  plants that were given the genetic formula  $WW^yii$  in Table 15. The  $F_1$  families from these crosses, which are listed in Table 17, show three color classes according to the expected 3 red : 1 yellow : 4 white ratio.

The fundamental relations of the factorial interactions are as follows:

- 3  $Wi$ , red.
- 1  $W^y i$ , yellow.
- 4  $\frac{1}{2} WI$ , white.
- 4  $\frac{1}{2} W^y I$ , white.

The progeny tests of the  $F_1$  plants from crosses Nos. 25 and 26 listed in Table 18 show that the four genotypes  $WW^yii$ ,  $WWii$ ,  $Wwii$ , and  $WW^yIi$  are present. The three progeny tests showing either a 1:3 or 3:13 ratio should account for one or both of the genotypes with the formulae  $WWIi$  and  $WwIi$ . The two expected genotypes,  $W^ywIi$  and  $W^ywi$ , did not appear among the small number of bulbs tested,

TABLE 17.—Data from crosses between heterozygous  $F_1$  red and white onion plants

Cross No.	Pedigree No.	Parental genotypes	F <sub>1</sub> progenies		
			Red	Yellow	White
25.....	2-1×8-2.....	$WwIi \times WW^{vi}i$ .....	4	1	6
26.....	7-2×2-2.....	$WW^{vi}i \times WwIi$ .....	5	0	6
Total.....			9	1	12
Theoretical, 3:1.....			8.25	2.75	11.0
Deviation.....			.75	1.75	1.0

$$\chi^2 = 1.271. \quad P = 0.54.$$

TABLE 18.— $F_2$  data from crosses Nos. 25 and 26

F <sub>1</sub> bulb No.	F <sub>1</sub> genotypic formulae	F <sub>2</sub> progenies		
		Red	Yellow	White
25a-1.....	$WWIi$ or $WwIi$ .....	13	0	23
25a-3.....	do.....	1	0	7
25-5.....	do.....	14	0	68
25-3.....	$WW^{vi}i$ .....	46	7	0
Theoretical, 3:1.....		39.8	13.2	0
Deviation.....		6.25±2.1		0
71-1.....	$WWi$ .....	21	0	0
Theoretical.....		21	0	0
71-2.....	$WwIi$ .....	242	0	85
Theoretical, 3:1.....		245.2		81.8
Deviation.....		3.25±5.3		
72-1.....	$WW^{vi}i$ .....	3	1	14
Theoretical, 3:1:2.....		3.4	1.1	13.5
Deviation.....		.4	.1	.5

$$\chi^2 < \text{than } 1. \quad P = \text{close fit.}$$

The three crosses between white and yellow listed below were made in an attempt to analyze the white and yellow relationships.

Cross No. 25, 2-1 ( $WwIi$ )×8-2 ( $WW^{vi}i$ ).

Cross No. 26, 7-2 ( $WW^{vi}i$ )×2-2 ( $WwIi$ ).

Cross No. 31, 2-2 ( $WwIi$ )×6-3 ( $W^{vi}W^{vi}i$ ).

It is evident that the white plants chosen for this analysis are white because they carry the inhibitor gene  $I$ . The tests resulting from these crosses show that the  $Ii$  factor pair holds the same relation to the gene for yellow,  $W^y$ , as it does for the gene for red,  $W$ . However, these crosses are not designed to give information as to the relationship between the gene for yellow,  $W^y$ , and the gene for white,  $w$ , which is necessary in order to demonstrate that the genes  $W$ ,  $W^y$ , and  $w$  form an allelomorphic series.

TABLE 19.—Data from a cross between a heterozygous  $F_1$  white onion plant and a homozygous yellow onion plant

Cross No.	Pedigree No.	Parental genotypes	F <sub>1</sub> progenies		
			Red	Yellow	White
31.....	2-2×6-3.....	$WwIi \times W^{vi}W^{vi}i$ .....	6	a 1	6
Theoretical, 1:1:2.....			3.25	3.25	6.5
Deviation.....			2.75	2.25	.5

$$\chi^2 = 3.95. \quad P = 0.14.$$

\* A questionable yellow bulb.

The  $F_1$  data presented in Table 19 involve a cross between the tested white  $F_1$  plant No. 2-2 (see Tables 2 and 10) that was given the genotypic formula  $WwIi$ , and a true-breeding yellow plant No. 6-3 that may be assigned the genotypic formula  $W^vW^vii$ , since on selfing it yielded 47 yellow bulbs. The poor fit observed in this case might have been influenced by errors in classification of the 13 bulbs, 5 were too small to insure accurate classification at harvest time. For this reason 7 of the 13 bulbs were grown in the greenhouse during the following winter months for the purpose of increasing their size so that an accurate classification could be made and seed production assured for the following year. While the bulbs were growing a reclassification was made in which one bulb was classified as a questionable yellow. It might be of interest to note that at the time a factorial hypothesis had not been formulated, which demanded a yellow class in this population. Only 3 of the 13 plants lived and produced seed the following year.

In self-pollinated progenies from the white  $F_1$  plants the ratios should be either 3 yellow:13 white or 3 red:1 yellow:12 white. The ratios in three  $F_2$  progenies recorded in Table 20 are obviously 3 red:1 yellow:12 white, resulting from  $F_1$  plants with the genotypic formula  $WW^vIi$ .

TABLE 20.— $F_2$  data from cross No. 31

F <sub>1</sub> bulb No.	F <sub>1</sub> genotypic formulae	F <sub>2</sub> progenies		
		Red	Yellow	White
31a-1.....	$WW^vIi$ .....	20	2	87
31-6.....	$WW^vIi$ .....	8	10	39
31b-1.....	$WW^vIi$ .....	26	11	103
Total.....		54	23	229
Theoretical, 3:1:12.....		57.4	19.1	229.5
Deviation.....		3.4	3.0	.5

$\chi^2=0.998$ .  $P$ = close fit.

A fairly large number of  $F_3$  progeny tests were made of the  $F_2$  individuals resulting from the selfed white  $F_1$  bulb 31-6. These  $F_2$  plants should include 9 different genotypes in the following proportions:

- 1  $WWII$ .
- 2  $WW^vII$ .
- 2  $WWIi$ .
- 4  $WW^vIi$ .
- 1  $W^vW^vII$ .
- 2  $W^vW^vIi$ .
- 1  $WWii$ .
- 2  $WW^vii$ .
- 1  $W^vW^vii$ .

The presence of six of these genotypes is definitely demonstrated by the  $F_3$  progeny results presented in Table 21. The three true-breeding white genotypes  $WWII$ ,  $WW^vII$ , and  $W^vW^vII$ , may be represented, although it is not possible to differentiate between them.

TABLE 21.— $F_3$  data from cross No. 31

$F_2$ bulb No.	$F_2$ genotypic formulae	$F_2$ progenies		
		Red	Yellow	White
404W-4.....	$WW^*ii$ .....	22	6	83
404W-6.....	.....do.....	28	9	113
404W-10.....	.....do.....	25	6	79
Total.....		75	21	275
Theoretical, 3:1 12.....		69.6	23.2	278.2
Deviation.....		5.4	2.2	3.2
404R-1.....	$WW^*ii$ .....	72	22	0
404R-2.....	.....do.....	53	18	0
Total.....		125	40	0
Theoretical, 3:1.....		123.75	41.25	0
Deviation.....		1.25±3.8		
404R-3.....	$WW^*ii$ .....	49	0	0
Theoretical.....		49	0	0
404Y-1.....	$W^*W^*ii$ .....	0	58	0
Theoretical.....		0	58	0
404W-1.....	$WW^*II$ or $WW^*iI$ or.....	0	0	200
404W-2.....	$WW^*II$ or $W^*W^*II$ .....	0	0	28
404W-11.....	.....do.....	0	0	338
Total.....		0	0	566
Theoretical.....		0	0	566
404W-3.....	$WW^*ii$ .....	93	0	271
404W-7.....	.....do.....	115	0	402
404W-8.....	.....do.....	38	0	101
404W-9.....	$WW^*ii$ or $WW^*iI$ .....	1	0	12
Total.....		246	0	774
Theoretical, 1:3.....		255	0	765
Deviation.....		9±9.4		
404W-5.....	$W^*W^*ii$ .....	0	45	99
Theoretical, 1:3.....		0	36	108
Deviation.....			9.0±3.5	

 $\chi^2=0.665$ .  $P$ =close fit.

\* One white set not included since it may have developed yellow color if allowed to mature.

b This progeny was not considered because it might be the result of either  $WW^*ii$  or  $WW^*iI$  genotype.

When a heterozygous red  $F_1$  plant from the original white and red cross,  $W_{22} \times R_{11}$ , is crossed with another heterozygous red  $F_1$  plant from a red and yellow cross the resulting population should be composed of the following genotypes in equal proportions:

$$\left. \begin{array}{l} 1 \text{ } WW^*ii \\ 1 \text{ } WW^*iI \\ 1 \text{ } W^*W^*ii \\ 1 \text{ } W^*W^*iI \end{array} \right\} 3 \text{ red.}$$

$$1 \text{ } W^*W^*ii \quad 1 \text{ yellow.}$$

The red and yellow proportions resulting from crosses Nos. 27 and 28 agree with the factorial analyses mentioned above. Progeny tests from the two crosses listed in Table 22 could not be made owing to storage-rot losses.

TABLE 22.—Data from crosses between heterozygous red  $F_1$  onion plants

Cross No.	Pedigree No.	Parental genotypes	$F_1$ progenies	
			Red	Yellow
27.....	3-2×8-2.....	$W^*wii \times WW^*ii$ .....	10	4
28.....	8-1×2-4.....	$WW^*ii \times W^*wii$ .....	13	2
Total.....			23	6
Theoretical, 3:1.....			21.75	7.25
Deviation.....			1.25±1.6	

## RELATION OF COLOR FACTORS TO DISEASE RESISTANCE

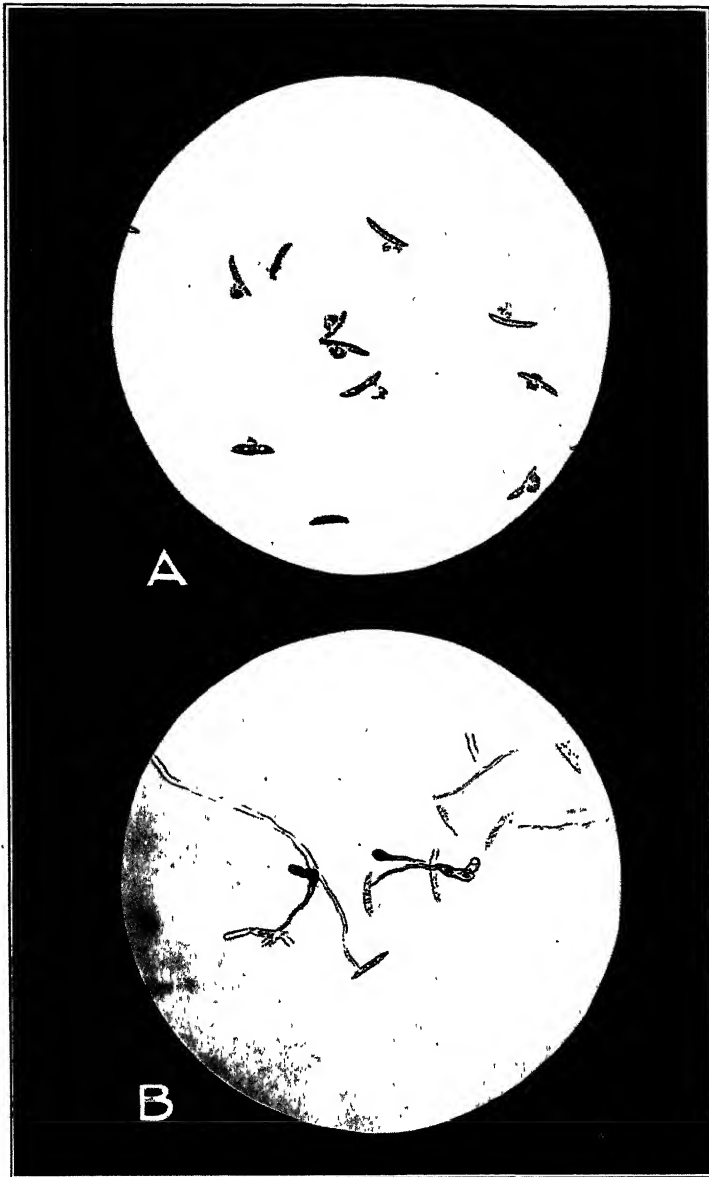
The pedigreeed stocks of onion bulbs discussed in this paper were subjected to field and laboratory tests to determine their resistance to the onion-smudge disease. As stated above, the field tests were not entirely satisfactory for the classification of bulbs into grades of resistance because of variation in the amount of inoculum and the influence of soil and environmental conditions upon infection. In general, however, the red-neck bulbs tended to show an intermediate stage between the high resistance of the red and the extreme susceptibility of pure white bulbs. The laboratory tests were used to determine the relative amounts of toxic material in the scales, which has been shown to be responsible for the resistance of red bulbs (5, 10, 12). The methods employed have already been described (p. 254).

When bits of dry red or yellow scales from either homozygous or heterozygous bulbs were placed in drops of spore suspension of *Colletotrichum circinans*, distinctly abnormal germination occurred. (See Pl. 3.) These abnormalities may be divided into three general classes: (1) Rupturing of the germ tube, characterized by a short ruptured germ tube from which a portion of the spore contents exudes in the form of a naked mass; (2) swelling of the spore; (3) thickening of the germ tube, characterized by a blunt thick-walled tube without an appressorium. Many spores failed to show changes in form, which might indicate that they were resistant to the toxic substances found in red scales. Drops of spore suspensions that had been treated with red scale tissue for 24 to 48 hours were shaken from the glass slide and several drops of a 1 per cent solution of dextrose were added to the smear. None of the normally shaped spores showed evidence of germination, whereas the controls, containing an additional lot of fresh spores, exhibited normal germination, showing that those which did not germinate had been killed by the toxic substances.

TABLE 23.—Data showing toxicity of dry outer scales of  $F_3$  onion bulbs from reciprocal crosses between red and white as related to smudge resistance

$F_3$ progeny No.	$F_3$ phenotype	$F_3$ ratio	Bulbs tested	Resistant	Intermediate resistant	Susceptible
234.....	Red.....	71 red : 218 white.....	18	18	0	0
234.....	Red neck.....	do.....	137	0	29	108
234.....	White and cream.....	do.....	50	0	4	46
Total.....			205	18	33	154
178.....	White and cream.....	47 white.....	25	0	0	25
191.....		374 white.....	25	0	1	24
202.....		326 white.....	25	0	0	25
206.....		14 white.....	12	0	0	12
221.....		604 white.....	7	0	0	7
224.....		132 white.....	25	0	0	25
227.....		30 white.....	25	0	0	25
231.....		105 white.....	32	0	2	30
246.....		90 white.....	25	0	1	24
255.....		40 white.....	25	0	2	23
259.....		83 white.....	25	0	1	24
Total.....			251	0	7	244

Red and white  $F_3$  bulbs from crosses Nos. 2 and 3 were tested for the presence of toxic materials in the outer dry scales. For convenience the tested bulbs presented in Tables 23 and 24 are grouped



EFFECT OF COLD WATER EXTRACT FROM DRY OUTER SCALES OF  
COLORED ONIONS UPON SPORE GERMINATION OF COLLETOTRICHUM  
CIRCINANS

A, Photomicrograph of spores about 17 hours after being placed in an extract from yellow scales. Note the masses of naked cytoplasm which have exuded from the spores after the tips of the germ tubes have ruptured. Compare with B; B, photomicrograph of spores about 17 hours after being placed in an extract from white scales. Note the normal germ tubes and appressoria. Compare with A.



empirically in the following classes: Resistant, intermediate resistant, and susceptible, according to the amount of toxic materials indicated by this spore-germination test. The individuals classified as intermediate resistant were tested twice to make certain that they contained materials which were toxic to the smudge organism. The bulbs that caused somewhat abnormal germ-tube development but allowed a high percentage of the spores to germinate and grow are included in the susceptible class.

In Table 23, the analysis of a progeny segregating for red and white shows that the proportion of intermediate resistant to susceptible bulbs in the red-neck class is approximately 1:4, whereas in the white-and-cream class it is about 1:12. In progenies breeding true for white, the proportion of intermediate resistant to susceptible is about 1:35. The data recorded in Table 24 also show a high correlation between the heterozygous red-neck character and intermediate toxicity. Approximately one bulb in three is partially resistant in the red-neck class, whereas in the white class the bulbs are all extremely susceptible. The three intermediate resistant bulbs occurring in a population of 20 cream bulbs are very deep cream in color, and may be the result of an interaction between the yellow gene  $W^y$  and the inhibitor gene  $I$ .

TABLE 24.—Data showing toxicity of white  $F_2$  bulbs from cross No. 31 between white and yellow onion plants as related to smudge resistance

$F_2$ progeny No.	$F_2$ phenotype	$F_2$ ratio	Bulbs tested	Intermediate resistant	Susceptible
301.....	Red neck.....	20 red . 2 yellow . 87 white.....	30	7	23
301.....	Cream.....	do.....	20	3	17
301.....	White.....	do.....	20	0	20

The data presented in Tables 23 and 24 indicate that white intermediate resistant bulbs occur more frequently in progenies segregating for red and white than in progenies breeding true for white. This relationship would be expected if the heterozygous factor pair  $Ii$  shows incomplete epistasis over the  $W$  and  $W^y$  genes (responsible for production of at least one substance toxic to the smudge organism), and the homozygous factor pair  $II$  usually shows complete epistasis. In other words, the appearance of a partially resistant class among white onion bulbs is undoubtedly due in a large measure to the presence in those bulbs of one of the resistant genes,  $W$  or  $W^y$ , but the expression of resistance by these genes is partially inhibited by the second factor pair  $Ii$ . The variability in the expression of the red-neck character should reflect a corresponding variation in the production of toxic materials. This relationship and the fact that the toxic substances are water soluble and subject to leaching explains, in part, how genetically white bulbs possessing heterozygous  $Ii$  factor pairs may vary in their reaction to disease. There is also the possibility of other modifying factors acting on the expression of disease resistance and on the expression of the heterozygous character, red neck.



## DISCUSSION AND CONCLUSIONS

Perhaps the most interesting contribution in this paper is the proof of the existence of two genes for resistance to smudge in onions, each having the ability to produce a definite water-soluble chemical entity, protocatechuic acid, which is extremely toxic to the causal organism, *Colletotrichum circinans*. Besides the production of a colorless water-soluble toxin, these two genes,  $W$  and  $W^y$ , are responsible for the production of red and yellow pigments, respectively, which contain the water-insoluble pigment, quercetin. It has been demonstrated (5) that upon alkaline fusion quercetin yields two toxic chemicals, oxalic acid and protocatechuic acid, and one nontoxic chemical, phloroglucinol. Oxalic acid probably does not contribute directly to the resistance of pigmented bulbs, for numerous attempts to isolate free oxalic acid from colored scales have failed.

Since the inheritance of disease resistance in this case involves the inheritance of certain pigments, any knowledge of the nature of disease resistance must of necessity be concerned with the relations between the chemistry and biochemistry of the plant pigments and the genetic factors for color inheritance.

The early investigations on the chemistry of plant pigments were carried out independently of the Mendelian point of view. Likewise, the first botanists and geneticists who turned their attention to the inheritance of color in plants failed to emphasize the fact that studies on the inheritance of color in reality involved studies on the inheritance of chemical compounds and chemical reactions. Wheldale (13, 14, 15, 16) was the first to point out this relationship and later Keeble and Armstrong (2, 3) and Keeble, Armstrong and Jones (4) added further details. The work published by Wheldale and Bassett (17, 18) on the pigment of the snapdragon (*Antirrhinum majus*) has shown yellow to be identical with the known substance luteolin, and ivory with the known substance apigenin. The pigment in rose doré was shown to be a red anthocyanin and the same pigment mixed with yellow gives the bronze color; similarly magenta contains a magenta anthocyanin, and this mixed with yellow gives crimson.

All the varieties may be expressed in terms of the following factors:

$Y$ —A factor leading to the production of luteolin in the lips and apigenin in the tube.

$I$ —A factor leading to the suppression of luteolin in the lips, apigenin being formed instead.

$L$ —A factor leading to the production of a tinge of red pigment in the lips.

$T$ —A factor leading to the production of a tinge of red pigment in the tube.

$D$ —A factor leading to the production of more anthocyanin pigment, i. e., a deepening or full-color factor.

$B$ —A factor converting red into magenta anthocyanin.

Thus the genes involved in flower colors in *Antirrhinum* can be expressed by: (1) The synthesis of definite chemical substances; (2) the modification of such substances once they are formed; (3) the control of enzyme action; (4) the modification of cell-sap reaction.

Onslow's (7) chemical interpretation of the interaction of the  $Y$  gene and the  $I$  gene in *Antirrhinum* may prove helpful in an elucidation of the chemistry of closely related pigments in the common onion that are governed by similar factorial interactions. The formula  $YYII$  has been assigned to the ivory-colored flower,  $YYii$  to the yellow-colored flower, and  $yyii$  (or  $II$ ) to the albino flower.

The *I* gene may be considered as responsible for the production of a substance that inhibits the formation of luteolin, or, since luteolin is more highly oxidized than apigenin, *I* may be a reducing agent. On treatment with caustic alkali apigenin forms *P*-oxybenzoic acid, whereas luteolin yields protocatechuic acid. Thus, an inhibitor gene may operate in *Antirrhinum* to control the formation of the organic acid, which, when present in the onion, contributes toward resistance to the smudge disease.

Neither the chemical nor the genetical work on onion pigments has shown how the toxic materials associated with color are formed. It is of course helpful to know that at least one toxic substance found in resistant bulbs can be produced as a decomposition product by alkaline fusion of the flavonol quercetin.

The question as to the mode of formation of the toxic materials in the outer scales of onion bulbs is of primary importance from the standpoint of breeding for disease resistance. From the evidence at hand it can be said that although the pigmented condition in the onion is closely correlated with resistance, the toxic substance which has been isolated is colorless. This suggests the possibility that toxins may be formed without a corresponding development of red and yellow pigments. The interaction of the incomplete inhibitor of color, gene *I*, with the genes for color, *W* and *W*<sup>y</sup>, lends support to this assumption. White bulbs with the genetic constitution *WWi* and *WwIi* show an intermediate toxicity to the disease. The bulbs possessing the heterozygous *Ii* factor pair and genes *W* or *W*<sup>y</sup> for color are neither red nor yellow but usually show light cream-colored outer scales. Occasional intermediate resistant white bulbs were observed in true-breeding white progenies, which indicates a possibility of obtaining resistant varieties of white onions.

In the light of our present knowledge the possibility of obtaining white resistant bulbs may be discussed from at least two different points of view:

1. If protocatechuic acid or other toxic substances are formed in resistant bulbs as precursors of the pigment quercetin then it is reasonable to expect that the *I* inhibitor gene in the presence of resistant color genes may permit the formation of toxins and suppress the formation of pigments. If, however, the toxins are decomposition products of the pigment quercetin, then it becomes difficult to conceive how white bulbs may possess resistance.

2. The *I* gene may interact with the color genes in such a manner as to produce a condition resulting in the production of a colorless isomer in the place of the yellow pigment quercetin. This would permit resistance in white bulbs even though the toxins were decomposition products. Willstätter (19) reports that many of the anthocyanins that are related to flavonols can readily be induced to change to colorless isomers. Whatever the nature of the systems producing toxins may be, it is more than likely that the complexes of chemical substances which produce the different expressions of disease resistance are the result of the activity of different "patterns of protoplasm," governed, in part at least, by the chromosomes.

Genetic analyses of disease resistance have been highly successful in those cases where the factorial interactions are manifested by the appearance of only two classes—resistant and susceptible. A factorial analysis of those cases where the reaction of the host to the parasite is evidenced by a range of types intermediate between

complete susceptibility and complete resistance is exceedingly difficult because of the necessity of employing arbitrary genetic classes. In this investigation the genetic formulae of the intermediate resistant plants are determined by the breeding behavior of the red, yellow, cream, and white characters. If the factors for disease resistance in the onion were not associated with color characters then it would not be possible to formulate an accurate genetic analysis of the intermediate resistant classes. A classification based solely on resistance tests would not indicate clearly the presence of an inhibitor of resistance gene. It is therefore of interest to note that, by the aid of color characters, it has been possible in this study to demonstrate one of the first cases of a satisfactory factorial analysis of intermediate resistance.

The data presented in this report have demonstrated that the various colored onions possess the following factors:

Red.....	<i>Wi.</i>
Yellow.....	<i>W<sup>v</sup>i.</i>
White.....	<i>wi</i> and <i>II.</i>
Red neck.....	<i>WIi.</i>

In progenies resulting from crosses between dominant white and red the presence of six of the nine different genotypes expected in a 13:3 ratio have been demonstrated, namely,

*WwIi.*  
*WW<sup>v</sup>Ii.*  
*Wwii.*  
*ww<sup>v</sup>Ii.*  
*wwii.*  
*WW<sup>v</sup>ii.*

In  $F_3$  progenies resulting from crosses between a white plant with the genetic formula *WwIi* and a yellow plant with the genetic formula *W<sup>v</sup>W<sup>v</sup>ii* the presence of six of the nine different genotypes expected in a 12:3:1 ratio have been demonstrated, namely,

*WWIi.*  
*WW<sup>v</sup>Ii.*  
*WWii.*  
*W<sup>v</sup>W<sup>v</sup>ii.*  
*W<sup>v</sup>W<sup>v</sup>Ii.*  
*W<sup>v</sup>W<sup>v</sup>ii.*

All of the genetic relations presented in this article strongly indicate that the genes *W*, *W<sup>v</sup>*, and *w* are members of an allelomorphous series. However, only two of the three critical tests necessary to demonstrate an allelomorphous relationship of three genes were made. In the absence of the inhibitor gene *I*, the gene *W* for red and the gene *w* for white show a simple monohybrid condition. Likewise gene *W* for red and gene *W<sup>v</sup>* for yellow show a simple monohybrid relationship. When the attempt was made to test out the yellow and white relationship the color-inhibiting gene *I* was not recognized, and white plants carrying this factor were used in the crosses. The final proof of the existence of a multiple allelomorphous color series in the onion depends, therefore, upon demonstrating the allelomorphism between *W<sup>v</sup>* and *w* genes.

#### SUMMARY

In an attempt to make a genetic analysis of the relation of the red, yellow, and white color classes to disease resistance in the common onion, data involving a classification of not less than 330 prog-

enies and approximately 22,000 individual bulbs are reported. An interpretation of the results obtained in the  $F_1$  to the  $F_4$  generations of the various genetic tests employed requires five different genes governing bulb pigmentation as follows:

- (1)  $I$  gene for incomplete inhibition of color.
- (2)  $i$  gene allowing expression of color.
- (3)  $W$  gene for red pigment.
- (4)  $W^y$  gene for yellow pigment.
- (5)  $w$  gene for white.

The inhibitor of color, gene  $I$ , is dominant to its recessive allelomorph  $i$ . The heterozygous factor pair  $Ii$  produces red-neck and cream-colored bulbs in the presence of the color genes  $W$  or  $W^y$ . Independent inheritance is demonstrated between the allelomorphic pair  $Ii$  and the genes for color.

The gene for red,  $W$ , is dominant to the gene for yellow,  $W^y$ , and the gene for white,  $w$ . It is assumed that the gene  $W$  is allelomorphic to the genes  $W^y$  and  $w$ . The relation between genes  $W^y$  and  $w$  has not been demonstrated.

The genes  $W$  and  $W^y$  are responsible for the production of a toxic substance, protocatechuic acid, in the outer scales of resistant onions.

The color-inhibitor gene,  $I$ , interacts with the gene for red,  $W$ , producing intermediate resistant bulbs.

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# EXTENSION OF PEARSON'S CORRELATION METHOD TO INTRAClass AND INTERCLASS RELATIONSHIPS<sup>1</sup>

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## INTRODUCTION

Because of the inadequacy of available data, the biometrician sometimes has to determine in terms of the universally comparable scale of the correlation coefficient the relationship between two variables, one of which is classed in two alternative categories only.

In such cases information concerning one variable, *A*, is recorded in multiple classes in terms of a quantitative scale or in multiple nonquantitative categories, while information for the other variable, *B*, is limited to the percentage of cases wherein *B* exceeds or falls short of a given intensity for each grade or class of *A*.

For the purposes of the present discussion such biserial tables are most conveniently thought of as originating from the condensation in one direction of the ordinary correlation or contingency surface.

A correlation surface having both *B* and *A* expressed in several quantitatively measured classes may be condensed by combining all the values of *B* into two alternative groups for each of the several classes of *A*. The result will be the biserial correlation table. Pearson (13)<sup>3</sup> showed how this relationship (biserial *r*) may be expressed in terms of the correlation scale.

In like manner a contingency surface having both *B* and *A* expressed in several nonquantitative categories may be condensed by combining all the values of *B* into two alternative groups for each of the several categories of *A*. The result will be the double-row contingency table. Pearson (14) has given methods for dealing with this relationship (biserial  $\eta$ ) also.

When the biserial correlation or contingency table is still further reduced by combining *A* as well as *B* in two alternative categories, the limit of condensation is reached in the (2×2)-fold table. Correlation and contingency coefficients have been derived from such tables in various ways. Pearson has expressed the relationship in terms of the equivalent-probability correlation coefficient (15).

Such extreme condensation<sup>4</sup> of the correlation or contingency surface as that which results when correlation or contingency tables with a reasonably large number of classes of both *A* and *B* are reduced to the biserial correlation or contingency distribution (and especially when such biserial distributions are further condensed from a (2×*n*)-fold to a (2×2)-fold distribution) must of necessity limit our knowl-

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<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 290.

<sup>4</sup> In the case of the contingency surface, and in the case of the correlation surface when treated by the correlation-ratio method, the grouping may be made too fine as well as too coarse to give trustworthy values of the coefficient. It is for this reason that "extreme" condensation is specified.

edge of the interrelationships between the two variables. The process is not indicated with any purpose of recommending it in cases in which the extended correlation or contingency surfaces are available, but merely to make clear the nature and the limitations of the biserial distributions as they must be considered in the present discussion.

The applicability of the correlation and contingency methods is not limited to the relationship between the magnitudes of  $A$  and  $B$  as measured on the same individual or on two individuals that are associated for some logical reason. In some cases, groups of individuals are associated in classes or in pairs of homologous classes, and it may be desirable to determine the direct intraclass correlation coefficient  $r_{A_1A_2}$  and  $r_{B_1B_2}$ , the cross intraclass correlation coefficient  $r_{A_1B_2}$ , or the direct,  $r_{A_1'A_2'}$  and  $r_{B_1'B_2'}$ , or cross  $r_{A_1'B_2'}$ , interclass correlation or contingency coefficients. Here the numerical subscripts denote a first and a second individual of the same class or of different but homologous classes, in the sense that each individual is used once as a first and once as a second member of a pair in the weighted correlation or contingency surface. The subscripts  $A$  and  $B$  without primes indicate individuals of the same class; those with primes denote individuals of different classes.

Such intraclass or interclass correlation or contingency tables may be readily formed by a procedure indicated elsewhere (2), or condensed<sup>5</sup> correlation tables (3) may be formed; finally, by employing the method of moments about 0 as origin (1), the intraclass and interclass coefficients may be calculated directly from the moments and product moments of the classes (4).

In a recent paper (10) attention has been called to the advantage of employing intraclass equivalent-probability correlation coefficients for certain cases in which the characteristics of the individual may be expressed in alternative categories only. Methods of determining the  $(2 \times 2)$ -fold tables for such coefficients from class moments have been given recently (8).

The purpose of the present paper is to consider methods whereby biserial intraclass and interclass coefficients may be determined directly from the moments of the classes and subclasses about 0 as origin.

#### THEORETICAL DEVELOPMENT OF METHOD, WITH PRACTICAL APPLICATION

Let a given lot of  $N$  individuals be divided for some logical reason into  $M$  classes. Let there be  $m$  subclasses in each class and  $n$  individuals per subclass. Further, let the  $n_s$  and  $n_d$  individuals comprising any subclass fall into the alternative categories,  $s$  and  $d$ ,<sup>6</sup> respectively.

The individuals of the  $M$  classes may be all that are available, in which case only the intraclass correlation can be determined. In other cases there may be another system (or other systems) of

<sup>5</sup> "Condensed" is here used in a sense somewhat different from that employed in reference to the biserial tables in the preceding paragraphs.

<sup>6</sup> Since biserial intraclass and interclass coefficients will probably be most frequently applied in cases in which the division is into the two classes "survived" and "died,"  $s$  and  $d$  may be used as symbols to denote the fate of an individual.

$M$  homologous classes, each divided into  $\mu$  subclasses of  $\nu_s + \nu_d = \nu$  individuals (of which  $\nu_s$  have the character  $s$  and  $\nu_d$  have the character  $d$ ) and comprising a total of  $\Sigma(\nu)$  individuals per class. In such a case the intraclass correlation for the variates of the second system of classes and the interclass correlation for the variates of the first and second systems can also be determined.

For any one of the  $n$  (or  $\nu$ ) individuals of any subclass only two alternative possibilities,  $s$  and  $d$ , may be realized. The other  $(m-1)$  (or  $\mu-1$ ) subclasses of the same class, or the  $\mu$  subclasses of the homologous classes of another system, are measured on a quantitative scale in terms of  $n_{sp}/n_p$ ,  $n_{sq}/n_q$ ,  $n_{sr}/n_r$ , etc., or in terms of  $\nu_{sp}/\nu_p$ ,  $\nu_{sq}/\nu_q$ ,  $\nu_{sr}/\nu_r$ , etc., for the  $p$ th,  $q$ th,  $r$ th, etc., subclass, or, if  $n$  or  $\nu$  be constant for all the  $m$  or  $\mu$  subclasses of the class, in the quantitative terms of  $n_{sp}$ ,  $n_{sq}$ ,  $n_{sr}$ , etc.,  $\nu_{sp}$ ,  $\nu_{sq}$ ,  $\nu_{sr}$ , etc., for these respective classes.

The problem now presented is twofold: (1) To determine the correlation between the fate of any individual in any subclass of a class, as expressed in alternative categories, and the characteristics of the  $(m-1)$  subclasses of the same class, expressed in quantitative terms of  $n_s/n$  or, if  $n$  be constant, of  $n_s$  (or in comparable values of  $\nu$ ). The result is the biserial intraclass correlation coefficient. (2) To determine the correlation between the fate of any individual of the  $n$  in a subclass of one system, as expressed in alternative categories, and the characteristics of the individuals of an homologous class of another system of classes, as measured in the quantitative terms of  $\nu_s/\nu$ , or, if  $\nu$  be constant, of  $\nu_s$ . The result is the biserial interclass correlation coefficient.

Since there are  $n$  individuals in a subclass, the arrays of values of  $n_s$ <sup>7</sup> associated with the alternative categories  $s$  and  $d$  into which the individuals of any subclass may be thrown must be given a total weighting of  $n$ . Individuals of character  $s$  will have associated with them the array of  $(m-1)$  subclasses weighted in an  $n_s$ -fold manner; individuals of character  $d$  will have associated with them the array of  $(m-1)$  subclasses weighted in an  $(n-n_s)$ -fold manner, or in an  $n_d$ -fold manner.

The problem may be clarified by an illustration. Table 1 gives the number of seedlings per hill (subclass) produced on 48 associated pairs of subplots, of 5 hills each, planted to sea-island and Durango cotton. This table represents an actual mapping of the data of experiment 2/25, plot D-2-4, of the United States Field Station, Sacaton, Ariz., summarized by Harris, Harrison, and Wadley (9, Table 8) and further discussed by Harris and Ness (10).

The symmetrical intraclass correlation surface for the sea-island variety is given as Table 2. The interclass correlation surface, measuring the relationship between the number of seedlings per subclass in each class of the sea-island variety and per subclass in each of the homologous classes or plots of the Durango variety, is given as Table 3.

<sup>7</sup> Quite obviously the arrays may be in terms of  $n_d/n$  instead of in terms of  $n_s/n$ , or in terms of  $n_d$  instead of  $n_s$ , if it is for any reason more desirable.



TABLE 1.—Number of seedlings per subclass (hill) on 48 adjoining pairs of subplots of sea-island and Durango upland cotton, together with the moments necessary for calculating biserial intraclass and interclass correlation coefficients based on data from cultures at the United States Field Station, Sacaton, Ariz., 1925 (experiment 2/25, plot D-2-4)

Sea-island cotton on—									Durango cotton on—								
Subplot No	Hill (subclass) No.					$\Sigma n.$	$\Sigma n_s^2$	$\Sigma n_s^3$		Hill (subclass) No					$\Sigma n_s$	$\Sigma n_s^2$	$\Sigma n_s^3$
	1	2	3	4	5					1	2	3	4	5			
4-1	4	1	6	5	5	21	103	531	5	6	5	4	4	24	118	594	
4-2	0	5	4	3	4	16	66	280	1	3	2	5	5	16	64	286	
4-3	0	0	0	0	0	0	0	0	1	0	0	4	5	10	42	190	
4-4	1	0	0	0	0	1	1	1	3	1	4	0	0	8	26	92	
4-5	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	
4-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4-8	5	6	0	0	0	11	61	341	0	0	4	3	4	11	41	155	
8-1	5	6	6	6	6	29	169	989	6	5	5	4	6	26	138	746	
8-2	4	3	6	5	4	22	102	496	4	3	6	4	2	19	81	379	
8-3	5	5	3	5	4	22	100	466	4	5	5	6	5	25	127	655	
8-4	6	3	5	6	6	26	142	800	6	3	5	6	4	24	122	648	
8-5	4	3	1	0	5	13	51	217	4	5	5	3	4	21	91	405	
8-6	5	6	6	2	5	24	126	690	3	6	5	4	6	24	122	648	
8-7	1	5	5	5	6	22	112	592	5	4	1	2	6	18	82	414	
8-8	3	2	5	1	1	12	40	162	4	4	3	5	6	22	102	496	
12-1	5	5	4	1	4	19	83	379	0	0	6	3	3	12	54	270	
12-2	3	5	3	2	0	13	47	187	0	0	0	3	0	3	9	27	
12-3	1	5	4	2	6	18	82	414	5	0	0	0	4	9	41	189	
12-4	3	4	1	3	4	15	51	183	1	4	5	3	3	16	60	244	
12-5	0	0	0	0	3	3	9	27	1	3	6	3	1	14	70	272	
12-6	0	0	0	0	5	5	25	125	0	0	0	1	3	4	10	28	
12-7	5	6	6	6	3	26	142	800	5	5	4	0	3	17	75	341	
12-8	5	4	6	5	6	26	138	746	5	5	5	4	3	22	100	496	
16-1	1	3	5	2	1	12	40	162	4	2	2	1	0	9	25	81	
16-2	3	4	5	5	5	22	100	466	4	4	0	4	3	15	57	219	
16-3	5	4	6	6	5	26	138	746	5	3	6	3	4	21	95	459	
16-4	5	6	6	2	0	19	101	565	4	5	4	4	5	22	98	442	
16-5	5	6	3	4	5	23	111	557	4	4	5	6	5	24	118	504	
16-6	4	3	5	6	2	20	90	440	1	5	5	4	3	18	76	342	
16-7	1	5	6	2	3	20	90	440	5	6	5	3	6	25	131	709	
16-8	3	6	6	4	6	25	133	739	5	5	5	4	5	24	116	564	
20-1	4	5	2	6	5	22	106	538	5	5	0	3	5	18	84	402	
20-2	6	5	3	2	4	20	90	440	2	5	4	6	5	22	106	538	
20-3	0	3	0	5	6	20	106	584	2	3	6	6	6	23	121	683	
20-4	5	4	6	2	5	22	106	538	6	1	4	4	5	20	94	476	
20-5	6	3	4	4	5	22	102	496	5	2	5	5	6	23	115	599	
20-6	4	2	5	4	6	21	97	477	5	4	2	5	1	17	71	323	
20-7	5	5	5	5	4	24	116	564	2	0	6	4	5	17	81	413	
20-8	4	5	5	4	0	18	82	378	6	2	1	4	2	15	61	297	
24-1	2	4	4	2	4	16	56	208	3	4	2	0	0	9	29	99	
24-2	0	3	0	0	0	3	9	27	2	3	1	0	0	6	14	36	
24-3	0	0	0	4	1	5	17	65	5	4	5	0	0	14	66	314	
24-4	2	4	1	0	1	8	22	74	4	1	0	0	0	5	17	65	
24-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
24-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
24-7	0	0	0	3	0	3	9	27	3	0	1	0	0	1	10	28	
24-8	0	0	0	0	0	0	0	0	0	2	0	0	0	2	4	8	
Total						715	3,371	16,957						699	3,151	15,231	

TABLE 2.—Intraclass correlation table for number of seedlings per subclass (hill) in sea-island cotton (experiment 2/25, plot D-2-4)

SEA-ISLAND									
								Total	
	0	1	2	3	4	5	6		
6	7	3	11	17	21	45	40	144	
5	14	13	15	22	43	48	45	200	
4	9	10	15	18	20	43	21	136	
3	17	7	7	4	18	22	17	92	
2	3	7	2	7	15	15	11	60	
1	10	6	7	7	10	13	3	56	
0	212	10	3	17	9	14	7	272	
Total	272	56	60	92	136	200	144	960	

TABLE 3.—Interclass correlation table for number of seedlings per subclass (hill) in associated classes of sea-island and Durango cotton (experiment 2/25, plot D-2-4)

		SEA-ISLAND						
		0	1	2	3	4	5	6
DURANGO	6	8	5	7	14	17	39	35
	5	17	11	17	28	47	66	64
	4	25	17	16	20	34	58	40
	3	31	8	8	20	23	29	21
	2	13	5	6	8	17	19	7
	1	37	7	5	9	11	12	4
	0	209	17	16	16	21	27	9
	Total	340	70	75	115	170	250	180
		Total						
		1, 200						

Such a symmetrical intraclass correlation surface as Table 2 may be condensed into a biserial distribution of arrays associated with the alternative categories into which any one individual of any subclass may be thrown. This may be done by combining either the columns or the rows (arrays) of frequencies which constitute the surface, after weighting each frequency of the array in an  $n_s$ -fold manner to form the arrays of  $n_s$  associated with  $s$  and in an  $(n-n_s)$ -fold manner (or in an  $n_d$ -fold manner) to form the arrays of  $n_s$  associated with  $d$  in the biserial distribution.

The results are given in Table 4. While it shows no superficial evidence of symmetry, this biserial distribution is fundamentally symmetrical in that each variate is used once as a first and once as a second variable. It is fundamentally asymmetrical in that the first variable is weighted and expressed in the alternate categories in which the individual seeds fall, whereas the second variable is expressed on the quantitative scale of number of seedlings per subplot.

TABLE 4.—Biserial intraclass correlation table for number of seedlings per subclass (hill) in sea-island cotton (experiment 2/25, plot D-2-4)

		SEA-ISLAND						
		0	1	2	3	4	5	6
SEA-ISLAND	S	215	164	233	317	515	791	625
	D	1, 417	172	127	235	301	409	239
	Total	1, 632	336	360	552	816	1, 200	864
		Total						
		5, 760						

The interclass correlation surface showing the relationship between the number of seedlings of sea-island and Durango cotton produced in the two associated (homologous) classes (Table 3) is not, and should not be made, a symmetrical table, since the two variables may be differentiated.

Since the two varieties may be differentiated, two biserial tables may be derived from this surface, one in which seeds of sea-island cotton are considered to fall in the two alternative categories,  $s$  and  $d$ , and the other in which the seeds of Durango cotton are classified in comparable alternative categories. Thus while the intraclass correlation surface for number of seedlings per subclass will give a unique value of the correlation coefficient, it is reasonable to expect that the two biserial distributions will give somewhat different values of the coefficient.

The weighting of the arrays of Table 3 as indicated above gives the two biserial distributions. (Tables 5 and 6.)

TABLE 5.—*Biserial interclass correlation table for number of seedlings per subclass (hill) in sea-island cotton, associated with alternative categories of Durango cotton of adjoining subplots (experiment 2/25, plot D-2-4)*

SEA-ISLAND								
DURANGO		0	1	2	3	4	5	Total
	S.....	389	194	232	389	587	933	3,495
	D.....	1,651	226	218	301	433	567	3,705
	Total.....	2,040	420	450	690	1,020	1,500	7,200

TABLE 6.—*Biserial interclass correlation table for number of seedlings per subclass (hill) in Durango cotton associated with alternative categories of sea-island cotton of adjoining subplots (experiment 2/25, plot D-2-4)*

DURANGO								
SEA-ISLAND		0	1	2	3	4	5	Total
	S.....	370	172	246	447	775	1,031	3,575
	D.....	1,520	338	204	393	485	469	3,625
	Total.....	1,890	510	450	840	1,260	1,500	7,200

The actual formation of the intraclass or interclass biserial table is unnecessary, since all that is required is  $s\bar{n}_s$  or  $d\bar{n}_s$ , and  $\bar{n}_s$ ,  $\sigma_{n_s}$ . Since  $s\sigma_{n_s}$ ,  $d\sigma_{n_s}$  may be desirable for other purposes, methods for their determination are given. Here the presubscript,  $s$  or  $d$ , denotes the character of the individual with which the weighted array of the value of  $n_s$ , upon which is based the mean or standard deviation, is associated. The absence of the presubscript indicates that  $\bar{n}_s$ ,  $\sigma_{n_s}$  are based on arrays weighted with all individuals (both  $s$  and  $d$ ) of the subclass with which they are associated.

#### CALCULATION OF BISERIAL INTRACLAS COEFFICIENTS FROM THE MOMENTS OF THE CLASSES

The moments for the array of the  $(m-1)$  values of  $n_s$  associated with any  $s$  of any one of the  $m$  subclasses of the class are

$$\begin{aligned} \Sigma(n_s) - n_s' & \text{----- i} \\ \Sigma(n_s^2) - (n_s')^2 & \text{----- ii} \end{aligned}$$

where  $\Sigma$  denotes the summation of all the values of  $n_s$  for the class and  $n_s'$  is the number of individuals of character  $s$  in the subclass with which the array of  $(m-1)$  subclasses is associated.

Since there are  $n_s$  individuals of character  $s$  and  $n - n_s = n_d$  individuals of character  $d$  in any subclass, i and ii must be weighted in an  $n_s$ -fold manner to obtain the moments for the weighted array associated with  $s$ , and in an  $(n - n_s)$ -fold, or in an  $n_d$ -fold, manner to obtain the moments for the weighted array associated with  $d$  for any subclass. Thus

$$n_s' \Sigma(n_s) - (n_s')^2 \text{----- iii}$$

$$n_s' \Sigma(n_s^2) - (n_s')^3 \text{----- iv}$$

are the moments for the weighted arrays associated with the fraction of individuals of any given subclass which have the character  $s$ , while

$$n_d' \Sigma(n_s) - n_d' n_s' = n \Sigma(n_s) - n n_s' - n_s' \Sigma(n_s) + (n_s')^2 \text{-----v}$$

$$n_d' \Sigma(n_s^2) - n_d' (n_s')^2 = n \Sigma(n_s^2) - n (n_s')^2 - n_s' \Sigma(n_s^2) + (n_s')^3 \text{-----vi}$$

are the moments for the weighted arrays associated with the fraction of individuals of any given subclass which have the character  $d$ .

Expressions iii and v give the first moments, while iv and vi give the second moments about 0 as origin for the weighted arrays associated with the individuals of any subclass of characters  $s$  (iii-iv) and  $d$  (v-vi), respectively.

But there are  $m$  such subclasses in any class and in consequence  $m$  values of iii-vi in any class.

Let  $S$  denote summation of the moments, weighted as indicated above, of the arrays associated with any subclass, and  $S$  denote summation for all the classes. For the whole sample iii and iv lead to

$$S\{S[n_s' \Sigma(n_s)]\} - S[S(n_s')^2] \text{-----vii}$$

$$S\{S[n_s' \Sigma(n_s^2)]\} - S[S(n_s')^3] \text{-----viii}$$

while v and vi lead to

$$S\{S[n_d' \Sigma(n_s)]\} - S[S(n_d' n_s')]$$

$$= S\{S[n \Sigma(n_s)]\} - S[S(n n_s')] - S\{S[n_s' \Sigma(n_s)]\} + S[S(n_s'^2)] \text{-----ix}$$

$$S\{S[n_d' \Sigma(n_s^2)]\} - S[S(n_d' n_s'^2)]$$

$$= S\{S[n \Sigma(n_s^2)]\} - S[S(n n_s')] - S\{S[n_s' \Sigma(n_s^2)]\} + S[S(n_s')^3] \text{-----x}$$

Since  $n_s'$  denotes merely any one of the  $m$  values of  $n_s$ , and  $S$  denotes summation for all values within one class,  $S(n_s') = \Sigma(n_s)$ , and  $S[n_s' \Sigma(n_s)]$  may be written  $[\Sigma(n_s)]^2$ . Similarly  $S[n_s' \Sigma(n_s^2)]$  becomes  $\Sigma(n_s) \Sigma(n_s^2)$ . These values are readily determined from frequency distributions of the number of individuals of character  $s$  per subclass.

Note further that for the class as a whole  $S(n_s')^2 = \Sigma(n_s^2)$  and  $S(n_s')^3 = \Sigma(n_s^3)$ , and that  $S[\Sigma(n_s^2)]$  and  $S[\Sigma(n_s^3)]$  are merely the second and third moments about zero as origin of the values of  $n_s$  for the whole sample, and can easily be calculated from a frequency distribution of  $n_s$ .

Now remembering that weighting is in an  $n(m-1)$ -fold manner, the mean,  $\bar{n}_s$ , and the standard deviation,  ${}_s\sigma_{n_s}$ , of the values of  $n_s$  associated with individuals of character  $s$  may be written

$$\bar{n}_s = \frac{S\{[\Sigma(n_s)]^2\} - S[\Sigma(n_s^2)]}{S\{S[n_s(m-1)]\}} \text{-----xi}$$

$${}_s\sigma_{n_s} = \frac{S[\Sigma(n_s) \Sigma(n_s^2)] - S[\Sigma(n_s^3)]}{S\{S[n_s(m-1)]\}} - \bar{n}_s^2 \text{-----xii}$$

In practical calculation it may be convenient to note that

$$S\{S[n_s(m-1)]\} = [\Sigma(n_s)](m-1)$$

Evaluation of these equations from the data in Table 1 gives, by way of illustration,

$$S\{[\Sigma(n_s)]^2\} = 14,717; S[\Sigma(n_s^2)] = 3,371$$

$$S[\Sigma(n_s)\Sigma(n_s^2)] = 71,421; S[\Sigma(n_s^3)] = 16,957$$

Whence, noting that  $S\{S[n_s(m-1)]\} = 2,860$ ;  $\bar{n}_s = 3.967133$ ,  $s_{n_s} = 1.818024$ , agreeing exactly with the constants calculated from the biserial distribution shown in Table 4.

The determination of  ${}_d n_s$  and  ${}_d \sigma_{n_s}$  proceeds in a similar manner

$${}_d \bar{n}_s = \frac{S[\Sigma(n_d)\Sigma(n_s)] - S[\Sigma(n_d n_s)]}{S\{S[n_d(m-1)]\}} \text{-----xiii}$$

or substituting for  $n_d$  its equivalent  $n - n_s$ ,

$$\begin{aligned} {}_d \bar{n}_s &= \frac{S[\Sigma(n - n_s)\Sigma(n_s)] - S\{\Sigma[(n - n_s)n_s]\}}{S\{S[(n - n_s)(m-1)]\}} \\ &= \frac{S\{\Sigma(n)\Sigma(n_s) - [\Sigma(n_s)]^2\} - S[\Sigma(nn_s) - \Sigma(n_s^2)]}{S\{S[mn - mn_s - n + n_s]\}} \\ &= \frac{S[\Sigma(n)\Sigma(n_s)] - S[\Sigma(nn_s)] - S[\Sigma(n_s)]^2 + S[\Sigma(n_s^2)]}{S\{S[mn - mn_s - n + n_s]\}} \text{-----xiv} \end{aligned}$$

If  $m$  and  $n$  both be constant, equation xiv may be written

$${}_d \bar{n}_s = \frac{S\{n(m-1)[\Sigma(n_s)]\} - S[\Sigma(n_s)]^2 + S[\Sigma(n_s^2)]}{(m-1)\Sigma(n - n_s)} \text{-----xv}$$

$${}_d \sigma_{n_s}^2 = \frac{S[\Sigma(n_d)\Sigma(n_s^2)] - S[\Sigma(n_d n_s^2)]}{S\{S[n_d(m-1)]\}} - {}_d \bar{n}_s^2 \text{-----xvi}$$

or substituting for  $n_d$  its equivalent  $n - n_s$ ,

$$\begin{aligned} {}_d \sigma_{n_s} &= \frac{S[\Sigma(n - n_s)\Sigma(n_s^2)] - S[\Sigma(n - n_s)n_s^2]}{S\{S[(n - n_s)(m-1)]\}} - {}_d \bar{n}_s^2 \\ &= \frac{S[\Sigma(n)\Sigma(n_s^2) - \Sigma(n_s)\Sigma(n_s^2)] - S[\Sigma(nn_s^2)] - \Sigma(n_s^3)}{S\{S[mn - mn_s - n + n_s]\}} - {}_d \bar{n}_s^2 \\ &= \frac{S[\Sigma(n)\Sigma(n_s^2)] - S[\Sigma(nn_s^2)] - S[\Sigma(n_s)\Sigma(n_s^2)] + S[\Sigma(n_s^3)]}{S\{S[mn - mn_s - n + n_s]\}} - {}_d \bar{n}_s^2 \text{-----xvii} \end{aligned}$$

If  $m$  and  $n$  both be constant, equation xvii may be written

$${}_d \sigma_{n_s}^2 = \frac{S\{n(m-1)[\Sigma(n_s^2)]\} - S[\Sigma(n_s)\Sigma(n_s^2)] + S[\Sigma(n_s^3)]}{(m-1)\Sigma(n - n_s)} - {}_d \bar{n}_s^2 \text{-----xviii}$$

The original data in Table 1 lead to

$$\begin{aligned} S\{n(m-1)[\Sigma(n_s)]\} &= 17,160 & S[n(m-1)\Sigma(n_s^2)] &= 80,904 \\ S\{[\Sigma(n_s)]^2\} &= 14,717 & S[\Sigma(n_s)\Sigma(n_s^2)] &= 71,421 \\ S[\Sigma(n_s^2)] &= 3,371 & S[\Sigma(n_s^3)] &= 16,957 \\ S\{S[n_d(m-1)]\} &= S[(m-1)\Sigma(n-n_s)] &= 2,900 \end{aligned}$$

and

$${}_d\bar{n}_s = 2.004828, {}_d\sigma_s = 2.257854$$

For the determination of the means and standard deviations of the whole population weighted as above indicated, the first and second moments on  $n_s$  may be obtained by adding the moments of  ${}_s n_s$  and  ${}_d n_s$ . Thus

$$\bar{n}_s = \frac{S[\Sigma(n) \Sigma(n_s) - \Sigma(nn_s)]}{S\{S[n(m-1)]\}} \text{-----xix}$$

$$\sigma_{n_s}^2 = \frac{S[\Sigma(n) \Sigma(n_s^2) - \Sigma(nn_s^2)]}{S\{S[n(m-1)]\}} - \bar{n}_s^2 \text{-----xx}$$

If  $m$  and  $n$  both be constant equations, xix and xx become

$$\bar{n}_s = \frac{S\{[n(m-1)][\Sigma(n_s)]\}}{(m-1) \Sigma(n)} \text{-----xxi}$$

$$\sigma_{n_s}^2 = \frac{S\{[n(m-1)][\Sigma(n_s^2)]\}}{(m-1) \Sigma(n)} - \bar{n}_s^2 \text{-----xxii}$$

For the present illustrative case the moments are already available and it is merely necessary to note that

$$(m-1) \Sigma(n) = S\{S[n_s(m-1)]\} + S[(m-1) \Sigma(n-n_s)] = 2,860 + 2,900 = 5,760$$

Thus  $\bar{n}_s = 17160/5760 = 2.979167$

$$\sigma_{n_s} = [(80904/5760) - (2.979167)^2]^{1/2} = 2.273851$$

agreeing exactly with the constants derived from the row of totals of Table 4.

With these values in hand the calculation of the biserial intraclass coefficient proceeds by the method of Pearson (13).

The constants for  $n_s$  have been given above;

$$\begin{aligned} {}_s\bar{n}_s &= 3.967133 \\ \bar{n}_s &= 2.979167 \\ {}_s\bar{n}_s - \bar{n}_s &= \bar{p} = +0.987966 \end{aligned}$$

Thus the mean number of seedlings per subclass is 0.99 higher in the subclasses associated with seeds of class  $s$  than in all the subclasses (hills) of the class ( ${}_s\bar{n}_s > \bar{n}_s$ ). Clearly, therefore, there is a positive

correlation between the survival of a seed in one subclass and the number of seeds surviving in associated subclasses.

Now the probability of seeds germinating is  $(2.979167)/6 = 0.496528$ . Hence in the notation of Sheppard's tables (17), represented by Pearson (16)

$$\frac{1}{2}(1 - \alpha) = 0.496528$$

$$\frac{1}{2}(1 + \alpha) = 1 - \frac{1}{2}(1 - \alpha) = 0.503472$$

For

$$\frac{1}{2}(1 + \alpha) = 0.50 \text{ and } \frac{1}{2}(1 + \alpha) = 0.51, \Delta = +0.003989 \text{ and } \Delta^2 = -0.000000$$

Hence

$$\theta = (0.503472 - 0.500000)/0.003989 = 0.870393$$

$$\frac{\theta(1 - \theta)}{2!} = \frac{0.870393 \times 0.129607}{2} = 0.056405$$

Determining  $z$  by the advancing difference formula

$$z = z_o + \theta \Delta_{z_o} - \frac{\theta(1 - \theta)}{2!} \Delta_{z_o}^2 + \dots$$

or numerically in the present case

$$z = 0.398942 - (0.870393 \times 0.000020) + (0.056405 \times 0.000040) = 0.398927$$

Finally, noting that for the whole series

$$\sigma_{n_s} = 2.273851$$

$$r = \frac{\bar{p}}{\sigma_{n_s}} / \frac{z}{\frac{1}{2}(1 - \alpha)} = \frac{0.987960}{2.273851} / \frac{0.398927}{0.496528} = 0.5408$$

The intraclass biserial correlation coefficient derived by an identical procedure from the data for Durango cotton given in the right side of Table 1 is  $r = 0.4284$ .

#### CALCULATION OF BISERIAL INTERCLASS COEFFICIENTS FROM THE MOMENTS OF THE CLASSES

The moments for the  $\mu$  values of  $\nu_s$  associated with any one of the values of  $n_s$  of the associated class of  $m$  values of  $n_s$  are  $\Sigma(\mu)$ ,  $\Sigma(\mu^2)$ . Weighting with the values of  $n_s$ , and denoting by  $S$  the summation for all  $m$  values of  $n_s$  and by  $S$  summation for all homologous classes

$$\bar{\nu}_s = \frac{S\{S[n_s \Sigma(\nu_s)]\}}{S[S(n_s \mu)]} = \frac{S[S(n_s) \Sigma(\nu_s)]}{S[\mu \Sigma(n_s)]}$$

$${}_s\sigma^2_{\nu_s} = \frac{S\{S[n_s \Sigma(\nu_s^2)]\}}{S[S(n_s \mu)]} - \bar{\nu}_s^2 = \frac{S[S(n_s) \Sigma(\nu_s^2)]}{S[\mu \Sigma(n_s)]} - \bar{\nu}_s^2 \text{-----xxiv}$$

Similar equations for  $d\bar{\nu}_s$ ,  $d\sigma_{\nu_s}$ ,  $\bar{\nu}_s$ , and  $\sigma_{\nu_s}$  are easily written. Obviously the alternative classification may be based on the  $\nu$  individuals of the subclasses of the classes of the second system, in which case the arrays required are those of  ${}_s n_s$  and  ${}_d n_s$  associated with individuals of class  $s$  and  $d$  of each value of  $\nu$ , or the constants required are  ${}_s \bar{n}_s$ ,  ${}_s \sigma_{n_s}$ ,  $d\bar{n}_s$ ,  $d\sigma_s$ ,  $\bar{n}_s$ ,  $\sigma_{n_s}$  all of which are readily obtained by a procedure similar to the above.

Two interclass coefficients may be obtained from the data of Table 1. Remembering that in this table  $n_s$  denotes number of seedlings per subclass (hill) in sea-island cotton, while  $\nu_s$  denotes the comparable values in Durango cotton, the interclass correlation for the two arrays per hill of Durango cotton associated with the two alternative classes of sea-island cotton of the adjoining plot may be based on the moments

$S[S(n_s)\Sigma(\nu_s)] = 13,464$   $S[S(n)\Sigma(\nu_s)] = 20,970$   $S[S(n)\Sigma(\nu_s^2)] = 94,530$   
whence, noting that  $S[\mu\Sigma(n_s)] = 3,575$ , and  $S[\mu\Sigma(n)] = 7,200$ ,

$${}_s \bar{\nu}_s = 3.766154 \quad \bar{\nu}_s = 2.912500 \quad \sigma_{\nu_s} = 2.155577$$

$$({}_s \bar{\nu}_s - \bar{\nu}_s) = (3.766154 - 2.912500) = +0.853654 = \bar{p}$$

The probability that the seeds of sea-island cotton with which the arrays of Durango cotton are associated will germinate and survive to the selected stage of development is, as in the preceding case (intraclass correlation, p. 288),

$$\frac{2.979167}{6} = \frac{3575}{7200} = 0.496528.$$

Hence, as before,  $z = 0.398927$ .

Finally

$$r = \frac{\bar{p}}{\sigma_{\nu_s}} / \frac{z}{\frac{1}{2}(1-\alpha)} = \frac{0.853654}{2.155577} / \frac{0.398927}{0.496528} = +0.4929$$

Similarly, the interclass correlation for the two arrays per hill of sea-island cotton associated with the two alternative categories of Durango cotton of the adjoining plot may be based on the moments

$S[S(\nu_s)\Sigma(n_s)] = 13,464$   $S[S(\nu)\Sigma(n_s)] = 21,450$   $S[S(\nu)\Sigma(n_s^2)] = 101,130$   
whence, noting that  $S[m\Sigma(\nu_s)] = 3,495$  and  $S[m\Sigma(\nu)] = 7,200$ ,  
 ${}_s \bar{n}_s = 3.852361$   $\bar{n}_s = 2.979167$   $\sigma_{n_s} = 2.273851$

$$({}_s \bar{n}_s - \bar{n}_s) = (3.852361 - 2.979167) = +0.873194 = \bar{p}$$

In determining the value of  $z$  it is necessary to base it on the alternative classes of Durango cotton with which the arrays of number of seedlings per subclass (hill) in sea-island cotton are associated. The probability of germination of Durango cotton is (p. 289)

$$\frac{\bar{\nu}_s}{6} = \frac{2.912500}{6} = \frac{3495}{7200} = 0.485417 = \frac{1}{2}(1-\alpha)$$



Interpolating as before, from Sheppard's tables (17),  $z = 0.398676$ . Finally

$$r = \frac{z}{\sigma_{n_s}} / \frac{z}{\frac{1}{2}(1-a)} = \frac{0.873194}{2.273851} / \frac{0.398676}{0.485417} = +0.4676$$

The difference between the two coefficients, which can not be expected to be identical, is only 0.0253.

### SUMMARY

Because of the inadequacy, or the innate limitations, of the available data, the biometrician frequently has to deal with variables classed in only two alternative categories. This has made necessary the development of correlation formulas suitable for dealing with  $(2 \times 2)$ -fold,  $(2 \times n)$ -fold, and  $(3 \times n)$ -fold tables.

Such conditions are encountered in investigations of seedling mortality, in which it may be necessary to class individual seeds in only two alternative categories—"died" and "survived."

In an earlier paper (10) the applicability of Pearson's equivalent-probability correlation coefficient to the measurement of the intraclass correlations between the fate of the individual seedlings of the same hill has been indicated. Such coefficients are essentially intraclass coefficients (4), since the seedlings in each hill are regarded as forming a separate class (or subclass, as it is necessarily designated here).

The present paper has considered the problem of the extension of Pearson's biserial correlation method to intraclass and interclass distributions.

It has been shown that both intraclass and interclass biserial correlation coefficients may readily be determined from the moments calculated from the original data.

While illustrations of the application of the method to the problem of the influence of field heterogeneity, in the sense in which this term has been used in earlier papers (5, 6, 7, 11, 12), on seedling stand in sea-island and Durango cotton have been given, the primary purpose of the present investigation has been to derive the necessary biometric formulas. An extended application of the method to the problem of the influence of field heterogeneity on seedling stand in cotton will be discussed later.

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# DEVELOPMENT OF BROWN CANCKER OF ROSES <sup>1</sup>

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## INTRODUCTION

The important rose disease known as brown canker, caused by *Diaporthe umbrina* Jenkins has been known in the United States for at least a quarter of a century.<sup>2 3 4</sup> The present known range of the disease includes most of the Atlantic Coast States, West Virginia, Kentucky, Pennsylvania, Alabama, Mississippi, Texas, and Michigan. Tea, hybrid tea, and hybrid perpetual roses are particularly susceptible. A few varieties in the following additional groups are also known to be affected: Pernetiana, Bengal or China, hybrid sweetbrier, dwarf polyantha, tea polyantha, Multiflora hybrid, Wichuriana hybrid, and Noisette hybrid. Rugosa, moss, and brier roses appear to be resistant or immune. Among the species on which the disease has been observed are the prairie (*Rosa setigera* Michx.), tea (*R. odorata* Sweet), dogbrier (*R. canina* L.), and cabbage (*R. centifolia* L.). The rose varieties on which the disease has been observed number about 175. Together with the rose species referred to above, these have already been listed.<sup>5 6</sup>

This account presents data pertaining to the development of brown canker and its causal fungus on stems, blossoms, and leaves of roses grown in the rose test garden at the Arlington Experiment Farm of the Department of Agriculture near Rosslyn, Va., in the Department of Agriculture grounds at Washington, D. C., and in the Van Fleet collection at the United States Plant Field Station at Glenn-dale, Md., between the years 1921 and 1927. The data are supplementary to those previously published,<sup>7</sup> and are intended to aid in the diagnosis of brown canker, which is the first step in control.

## STEM CANCKER

Plate 1, A-C, shows initial infection lesions <sup>8</sup> on a plant of Gloire Lyonnaise on October 17, 1923. The first two stems represent the current year's growth and the third stem the growth of the preceding year. This was a control plant for a certain spraying experiment. Although search had been made, no evidence of initial infection had been found on current-year stems until early in August. At that time there were seen only two small lesions on a single stem; these were at first purple, later turning white. On one (pl. 1, A) of

<sup>1</sup> Received for publication Oct. 13, 1930; issued March, 1931.

<sup>2</sup> JENKINS, A. E. BROWN CANCKER OF ROSES CAUSED BY *DIAPORTHE UMBRINA*. Jour. Agr. Research 15: 593-600, illus. 1918.

<sup>3</sup> ——— BROWN CANCKER OF ROSES. Mycologia 17: 87-88. 1925.

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<sup>5</sup> JENKINS, A. E. Op. cit. (Excerpt of publication cited in footnote 4, pp. 182-183.)

<sup>6</sup> MARTIN, G. H., and JENKINS, A. E. PRELIMINARY LIST OF FUNGI AND DISEASES OF ROSES IN THE UNITED STATES. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rpr. Sup. 63. 354-369. 1928. [Mimeographed.]

<sup>7</sup> See footnotes 2, 3, and 4.

<sup>8</sup> Jenkins, A. E. Op. cit. (See footnote 4.)

the two stems representing current year's growth, purple spotting, or initial infection lesions, became visible about October 4 and on the other (pl. 1, B) approximately on September 10. In three weeks those on the second or larger stem had begun to turn white. The initial infection lesions on the older stem (pl. 1, C) were formed during the growing season of 1922 when that stem developed. Remaining quiescent during the succeeding season when advance from such lesions commonly takes place, they had as yet caused no appreciable injury to the stem. As determined by tissue cultures made from them, the fungus appeared to be as readily viable in these older lesions as in those on the current-year stems. In making the isolations small bits of diseased tissue were used as plantings. These were first dipped for about 30 seconds in a solution of mercuric chloride (1:1,000) and then thoroughly rinsed in sterile water. Numerous plantings were made from each stem, some from lesions grouped above the leaf scars and others from those scattered about on the stems. The colonies were practically all pure for *Diaporthe umbrina*. (Fig. 2, C.) A few were of other fungi common on rose, namely, *Coniothyrium fuckelii* Sacc., *Gloeosporium* sp., and *Alternaria* sp. Pycnidia of *Coniothyrium fuckelii* have occasionally been found on whitish stem lesions which were apparently only those of brown canker. Whether this organism may also initiate such lesions has not been determined.

The illustrations in Plate 1, D-G, represent the rapid spring advance of cankers from initial infection lesions on 1-year-old stems of La Tosca (pl. 1, D) and of Gloire Lyonnaise (pl. 1, E and F) and from a larger canker on what is probably a 2-year-old stem (pl. 1, G). This material was gathered late in March, 1924. The lower as well as possibly the upper canker on the older stem apparently developed during the previous growing season. Here the fungus may have entered the stem through a blossom spur or a wound. The noticeable black specks in the newly formed cankers are pycnidia which have not yet broken through the epidermis. Conidia were already present in them. The fresh stems were placed in an iced refrigerator with their lower cut ends in water. After one week all of the cankers had advanced considerably. Conidia were then being exuded from the dark-colored pycnidia, which had ruptured the epidermis in the region of the lenticels. Perithecia containing immature asci and ascospores had also begun to form in the new cankers, but their presence could be detected only through microscopic examination.

The cankered stems shown in Figure 1 are representative of a condition which is often called "die-back."<sup>9</sup> When first observed on February 5, 1924, the stem of Col. R. S. Williamson (fig. 1, A) was entirely cankered to within 2 inches of a point near the base where earth had been mounded around it for winter protection. The fresh coloring of the diseased bark indicated that the die-back was fairly recent and that the fungus was then active. When photographed on March 17 the canker had advanced to, but not beneath, the surface of the soil; in some manner the earth may have acted as a barrier to its further downward progress. The La Tosca stem (fig. 1, B) is from a control plant employed in the spraying experi-

<sup>9</sup> Linz, C. L. ROSE LOVERS AT THE NATION'S CAPITAL. Florists' Exch. 55: 1615, 1638. 1923.



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A-C.—Initial infection lesions of brown canker on current year (A and B) and 2-year-old (C) stems of Gloire Lyonnaise.  $\times 1$ .

D-G.—Stems of La Tosca (D and G) and Gloire Lyonnaise (E and F), showing early spring advance of brown canker from initial infection lesions on growth of the previous year and from large cankers on that probably 2 years old (G).  $\times 1$ .

(Color drawings by J. Marion Shull)

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FIGURE 1.—Die-back due to brown canker on stems of (A) Col. R. S. Williamson, (B) La Tosca, and (C) Gloire Lyonnaise, collected in March (A and C) and in October (B).  $\times 1$



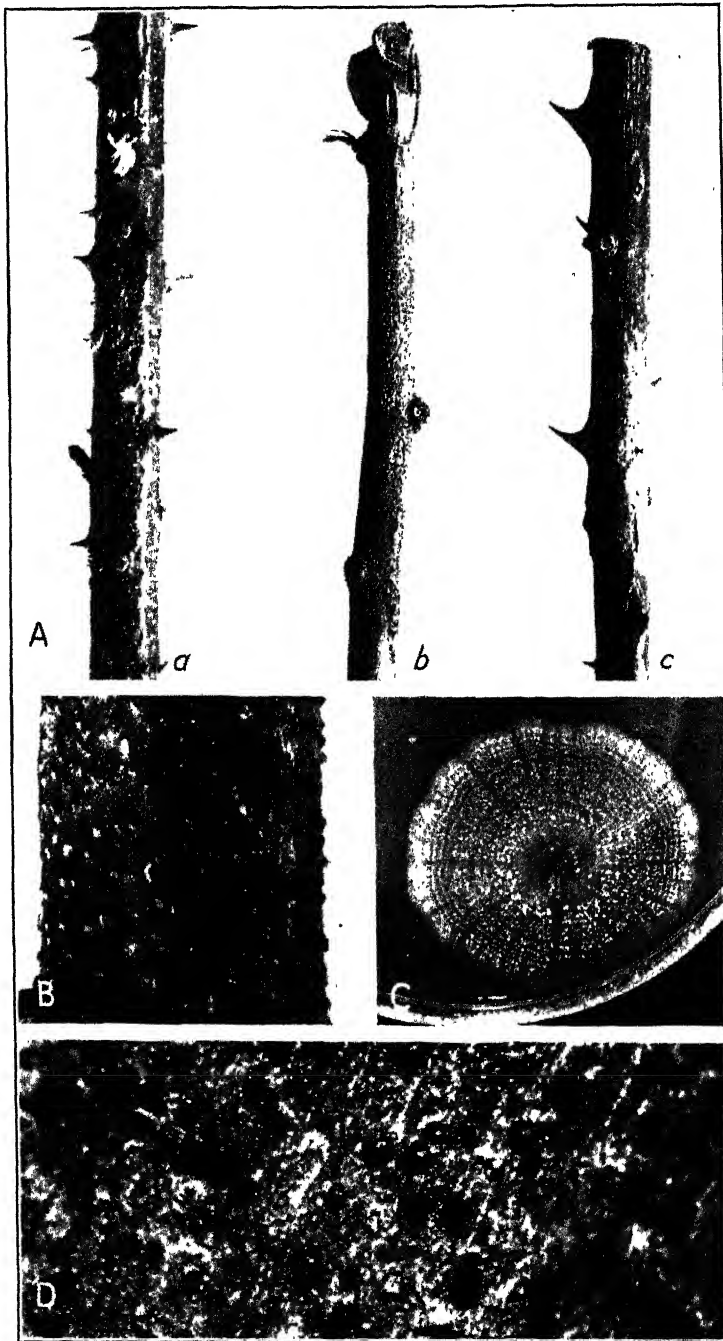


FIGURE 2.—A, Perithecial pustules of *Diaporthe umbrina* on rose stems collected in winter (a), summer (b), and autumn (c).  $\times 1$ . B, Enlargement of part of canker shown in A, b.  $\times 6$ . C, Sixteen-day-old isolation of *D. umbrina* on corn-meal agar medium in Petri dish, from tissue planting of initial infection lesions on stem shown in Plate 1, B.  $\times 1$ . D, Pycnidia of *D. umbrina* on old dead stub of Killarney Queen.  $\times 25$

ment conducted at the Glendale station during the growing season of 1926.<sup>10</sup> Collected on October 8, it illustrates die-back from blossom infection occurring during summer and autumn on current season's growth, such manifestation of brown canker having been practically controlled in the sprayed plants. Die-back on the

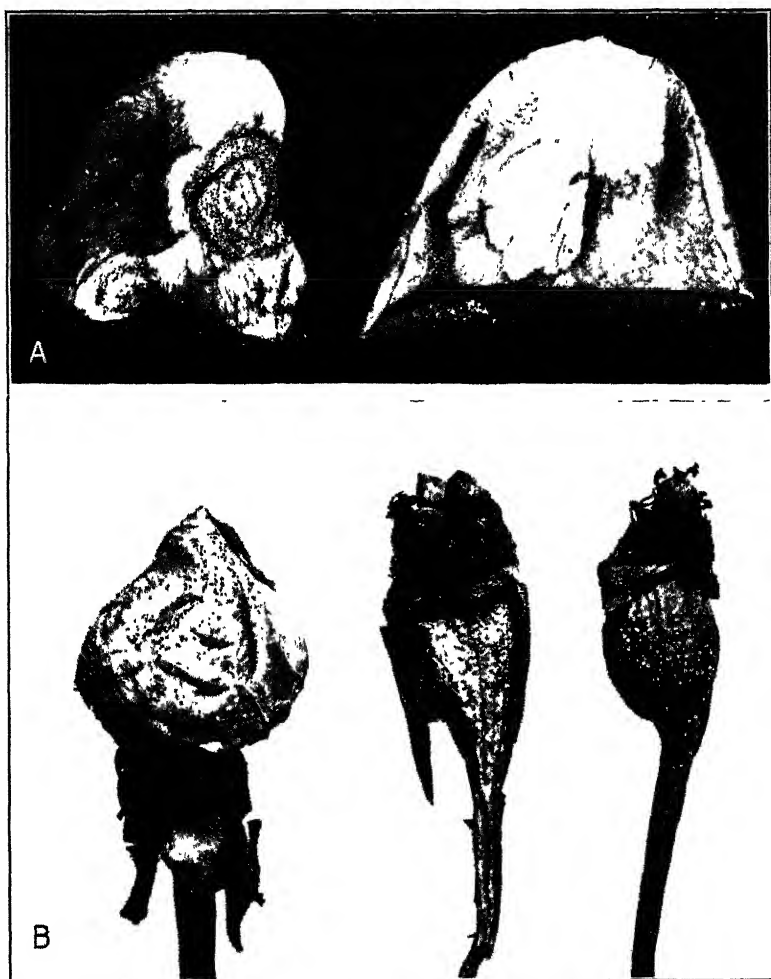


FIGURE 3.—A, Two fresh petals of Red Radiance infected by *Diaporthe umbrina*, on one of which are numerous pycnidia.  $\times 14$ . B, Pycnidia of *D. umbrina* as present in June on unopened blossom and hips of a climbing hybrid tea rose which grew late the previous autumn.  $\times 2$

2-year-old stem of Gloire Lyonnaise (fig. 1, C) collected on March 25, 1924, occurred largely during the previous season.

The perfect stage of the fungus was present on all three of the cankered stems in Figure 2, A; part of the central stem is enlarged in Figure 2, B. The first stem, one of *Rosa corymbulosa*, was observed in January, 1924. It appeared to have been recently killed

<sup>10</sup> JENKINS, A. E. Op. cit. (See footnote 4.)

by the fungus, which was fruiting over practically its entire surface. The second and third stems were collected in June, 1924, and October, 1923, respectively. This material is illustrative of the fact that in the vicinity of Washington, D. C., the perfect stage of the fungus may be found during the autumn, winter, and summer, as well as during the spring of the year.<sup>11</sup> Old and unusually large pycnidia, each containing many conidia, are seen near the dead cut end of the stem of Killarney Queen shown in Figure 2, D. The falling away of the epidermis has left the pycnidia thus exposed.

### BLOSSOM INFECTION

Fresh petals of Red Radiance affected by the brown-canker disease are shown in Figure 3, A. Infection is indicated in the first petal

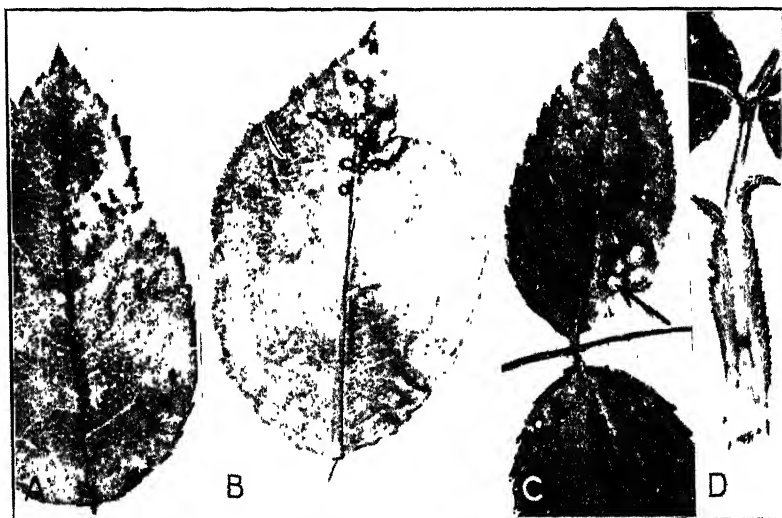


FIGURE 4.—Leaves of Gloire Lyonnaise and Oakmont showing leaf spot due to *Diaporthe umbrina*: A, Purple spotting; B, and C, larger or smaller lesions which are white or cinnamon-buff color at the center; D, petiole and stipule infection.  $\times 13\frac{1}{4}$

only by the cinnamon-buff<sup>12</sup> discoloration characteristic of many lesions of brown canker, whether occurring on blossoms, stems, or leaves; in the other petal, pycnidia of the causal fungus are present in great numbers and are arranged in well-defined concentric circles, as often occurs in this species. (Fig. 2, C.) These petals, as well as the dead unopened blossom and two dead rose hips of a hybrid tea variety shown in Figure 3, B, were collected in June, 1921. The pycnidial stage of the fungus is also fruiting in abundance on the dead bud and hips, these having developed late the previous autumn.

### INFECTION ON LEAVES

The leaf spot resulting from infection by *Diaporthe umbrina* is sometimes difficult to distinguish from other rose leaf spots. Recently formed purple spots or those that have remained purple are shown in Figure 4, A, and spots whose central regions have later

<sup>11</sup> JENKINS, A. E. Op. cit. (See footnote 4.)

<sup>12</sup> RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

become white or cinnamon buff in Figure 4, B and C; a pycnidium has formed at the center of one of the small lesions. (Fig. 4, C, arrow.) Petiole and stipule infection are illustrated in Figure 4, D.

#### SUMMARY

This paper deals with the development of brown canker of the rose and its causal fungus, *Diaporthe umbrina*, on stems, blossoms, and leaves of roses grown in the vicinity of Washington, D. C. The data presented are supplementary to those previously published or are more detailed explanations of them. Several aspects of the disease and fungus are described.



# CONTROL OF MOISTURE CONTENT OF AIR AND WOOD IN FRESH-AIR CHAMBERS<sup>1</sup>

By IRA HATFIELD

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## NEED FOR IMPROVED METHOD

Many pathological and physiological investigations call for a method of controlling the relative humidity of chambers in which experiments are to be conducted for a long period. Sulphuric acid solutions have been used extensively for such control, but the density of the solutions must be maintained definitely, and exchange of air in the chambers is greatly handicapped, if not entirely impeded. Criticism has also been made concerning the fumes that occur when this acid is used in the humidifiers. These fumes have received little attention as yet from those who have used the sulphuric-acid method.

In attempting to control the moisture content of wood blocks in an investigation of the moisture requirements of wood-inhabiting fungi, the writer needed an improved method of controlling the relative humidity. The features particularly desired were (1) a continuous supply of fresh air, (2) the maintenance of controlled relative humidity in culture chambers for indefinite periods of time, (3) freedom from contamination by microorganisms throughout the entire experiment, and (4) a means of collecting the respiratory products for use as an index of physiological activity.

## SALT SOLUTIONS AND RELATIVE HUMIDITY

It is known that a saturated solution of a salt in contact with an excess of that salt will maintain a certain fixed relative humidity in an atmosphere confined above it, provided the temperature remains constant<sup>3</sup> and adequate air circulation is provided. Use was made of this fact in the present investigation through the selection of salt solutions having vapor tensions that would maintain the desired relative humidities.

A few of the salts used for this purpose are listed in column 1 of Table 1; column 2 gives the corresponding temperature ranges for the relative humidities as recorded in column 3, the values of which have been taken from the International Critical Tables.<sup>4</sup> A glance at column 3 reveals that there is enough difference in the relative humidity values for the salts to make a useful graded series. By using other salts listed in the critical tables a complete series from 2 per cent to 98 per cent humidity could be obtained.

<sup>1</sup> Received for publication Oct. 14, 1930; issued March, 1931.

<sup>2</sup> In cooperation with the Forest Products Laboratory, Forest Service, U. S. Department of Agriculture.

<sup>3</sup> SPENCER, H. M. LABORATORY METHODS FOR MAINTAINING CONSTANT HUMIDITY. *In* International Critical Tables . . . 1: 67-68. New York and London. 1926.

<sup>4</sup> SPENCER, H. M. *Op. cit.*

TABLE 1.—Salts used for regulating the relative humidity in culture chambers and the moisture content of wood exposed to these relative humidities

Salt	Temperature	Relative humidity	Equilibrium moisture content of southern yellow pine, based on oven-dry weight	
			Expected <sup>a</sup>	Found <sup>b</sup>
	° C <sup>c</sup>	Per cent	Per cent	Per cent
Pb(NO <sub>3</sub> ) <sub>2</sub> .....	20	98	( <sup>c</sup> )	29.0
NaBrO <sub>3</sub> .....	20	92	24.0	24.0
K <sub>2</sub> CrO <sub>4</sub> .....	20	88	21.7	21.7
KBr.....	20	84	19.9	19.7
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	20-30	81-81.1	18.7	18.7
NH <sub>4</sub> Cl.....	20-30	79-79.5	18.1	18.0
NaClO <sub>4</sub> .....	20	75	16.8	16.7
NH <sub>4</sub> (Cl+KNO <sub>3</sub> ) <sup>d</sup> .....	20-30	72.6-68.6	15.3	15.3
Mg(C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> ) <sub>2</sub> ·4H <sub>2</sub> O.....	20	65	14.0	14.0
NaBr·2H <sub>2</sub> O.....	20	58	12.5	12.4

<sup>a</sup> Obtained by averaging the two sets of values for southern yellow pine listed in Technical Note F-13 of the Forest Products Laboratory. This note also gives values for other species.

<sup>b</sup> Values obtained for *Pinus palustris* over a period of 14 weeks.

<sup>c</sup> Not given

<sup>d</sup> Equal proportions of the 2 salts were used.

<sup>e</sup> At 70 per cent.

The usual temperature fluctuations in the laboratory in which this investigation was carried out are within the range between 20° to 30° C., inclusive. The table shows that it is possible to find salts that will give approximately the relative humidity values desired and at the same time maintain the individual values under usual laboratory temperature fluctuations. Further work on this phase of the problem is in progress.

### APPARATUS FOR REGULATING RELATIVE HUMIDITY

The equilibrium moisture content of wood varies slightly from species to species. It seemed advisable, therefore, to check the moisture content definitely for a single species exposed to the specific conditions necessary in most pathological investigations. Accordingly, specimens of one of the southern yellow pines (*Pinus palustris* Miller) were placed in an apparatus based in principle upon that used by Heck <sup>5</sup> for soil work. Figure 1 presents a diagram of the apparatus. By means of an aspirator air is drawn through the intake tube containing soda lime. The chemicals remove the carbon dioxide and some of the water from the air. From this tube the air passes into a tower filled with pumice stone of such size as to permit its passage through a screen having four meshes to the inch. The stone has been treated with sufficient sulphuric acid to wet it thoroughly without allowing an excess of the acid to collect in the bottom of the tower. The sulphuric acid dries the air further and also aids in removing such laboratory gases as ammonia. The air then passes through another tower containing soda lime to assure that all the gases, including any acid fumes, have been removed. Any spores or organisms in the air are removed by conducting it through a U tube containing sterile cotton. The air is then bubbled through two Erlenmeyer flasks containing the salt solutions chosen to give the desired

<sup>5</sup> HECK, A. F. A METHOD FOR THE DETERMINATION OF TOTAL CARBON AND ALSO FOR THE ESTIMATION OF CARBON DIOXIDE EVOLVED FROM SOILS. Soil Sci. 28: 225-233. 1929.

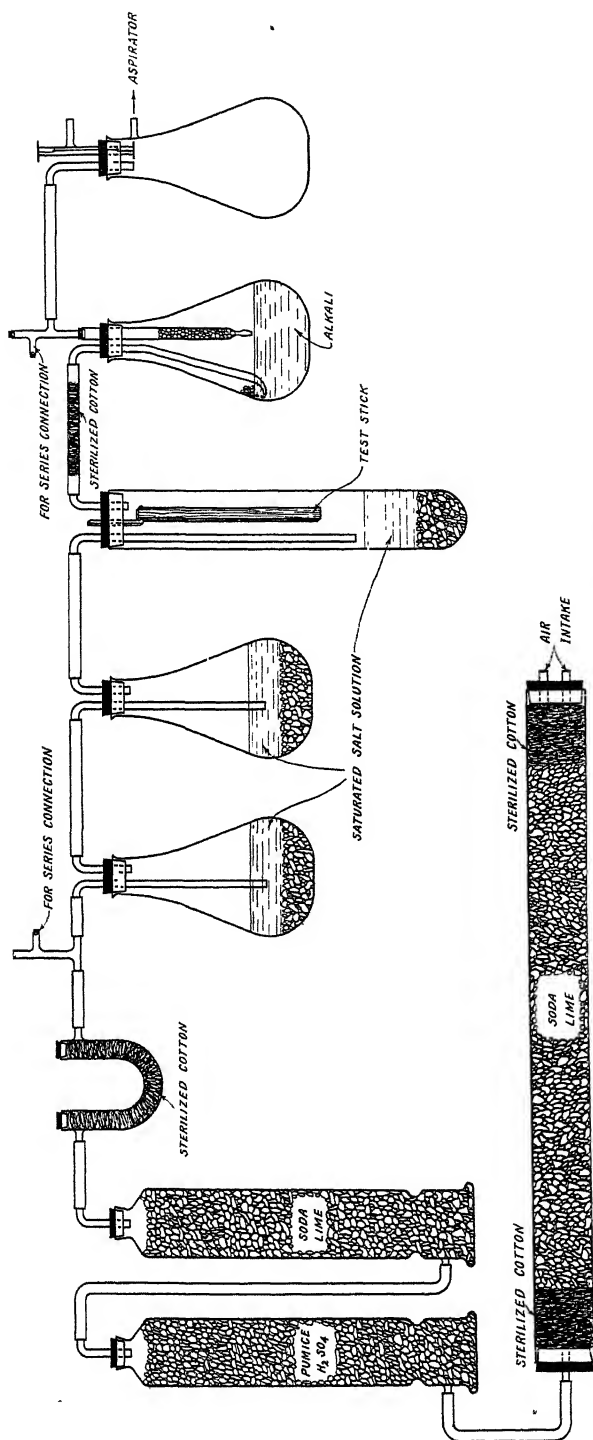


FIGURE 1.—Apparatus for regulating relative humidity



relative humidity. This brings it to the proper moisture content. In the culture chamber is more of the salt solution, to aid in maintaining the proper relative humidity. In this chamber the air is not bubbled into the solution, but is drawn in close to the surface of the liquid, which prevents spraying of the solution on to the test stick. The test stick is suspended on a bent glass rod, thus avoiding the use of a corrodible metal hanger.

The air from the culture chamber is conducted into a carbon-dioxide trap constructed as described by Heck<sup>6</sup> and as represented in Figure 1. As the air is drawn from the culture chamber it passes through a rubber-tubing connection that is filled with cotton. The cotton in the tubing serves only as a means of keeping contamination from entering the culture chamber when the carbon-dioxide trap is removed for titration purposes. From the cotton-filled tubing the air passes through the entrance tube to the carbon-dioxide trap. This entrance tube is drawn out at its lower end and bent toward the side of the flask to make the bubbles adhere to the side of the flask, thus affording greater diffusion of carbon dioxide into the water before the bubbles finally burst. The exit of air from the trap is made through a bead tower, where any remaining traces of carbon dioxide can be absorbed by the capillary film of alkaline solution around each bead. The bead tower is made by drawing out a  $\frac{3}{4}$ -inch glass tube and so flattening the constriction that two beads will lie at the bottom of it, with enough space remaining to allow a free passage of fluid either way. This facilitates an easy and complete washing of the tower and the solid glass beads when titrations are to be made. The use of this carbon-dioxide trap makes possible the collection of the carbon dioxide given off by any fungus that may be growing on or in the test stick. Measurement of the carbon dioxide by titration gives curves that may be used as an indication of the growth of the fungus at the given moisture content.

The chief purpose of the final flask shown in Figure 1 is to permit the attachment of a vacuum gauge and an aspirator. Between this flask and the U tube are the four chambers last described, and on each side of the four is a T connection, shown with its free ends closed. A series of groups of chambers, four in each group, may be connected between these T's. Thus a whole battery of culture chambers can be kept at graduated humidities with air drawn from the same towers. For different humidities it is necessary to duplicate only the part of the apparatus shown between the points marked "for series connections" in Figure 1.

When several tests are set up in parallel the rate of air movement through the chambers can be distributed evenly by means of stop-cocks applied either at the rubber connection previous to the first Erlenmeyer flask containing the saturated salt solution, or at the rubber connections between the carbon-dioxide titration chamber and the tube leading to the aspirator. Uniform rapid air exchange is desirable, as it keeps the moisture gradient of the stick more nearly uniform and assures complete removal of carbon dioxide as fast as it is produced.

Column 5 of Table 1 shows the fine gradient obtained from the various salts when they were used to control the moisture content of

<sup>6</sup> HECK, A. F. Op. cit.

the test sticks placed in the various culture chambers in a consecutive series of humidities. The experimental results for the one species examined agree very well with the equilibrium moisture content values for southern yellow pine presented in Technical Note F-13 of the Forest Products Laboratory<sup>7</sup> and as averaged by means of curves and tabulated in column 4 of Table 1. This agreement means that the test sticks dried down to the equilibrium moisture content expected for each value of relative humidity, and were maintained at that moisture content for several months within satisfactorily small limits of experimental error. It was necessary, of course, to keep each test stick in its culture chamber long enough to insure the attainment of equilibrium with the moisture conditions of the atmosphere surrounding it. Mention should also be made of the fact that certain salts, otherwise satisfactory for controlling humidity, can not be used when carbon-dioxide tests are to be made.

Although the apparatus described was designed especially for studies of the growth of wood-destroying fungi, it could easily be adapted for spore-germination or seed-germination tests, the culture of small plants, and perhaps for other purposes. Where experiments with Petri-dish cultures or other cultures are to be made, it would be necessary only to modify the culture chamber diagrammed in Figure 1. This modified chamber might take the form of a special vacuum desiccator or a culture chamber similar to the one used by Fellows<sup>8</sup> for determining the influence of oxygen and carbon dioxide on the growth of *Ophiobolus graminis* in pure culture.

<sup>7</sup> UNITED STATES DEPARTMENT OF AGRICULTURE, FOREST SERVICE, FOREST PRODUCTS LABORATORY. MOISTURE CONTENT OF WOOD AT DIFFERENT HUMIDITIES. U. S. Dept. Agr., Forest Serv., Forest Prod. Lab. Tech. Note F-13, 2 p. 1919. [Mimeographed]

<sup>8</sup> FELLOWS, H. THE INFLUENCE OF OXYGEN AND CARBON DIOXIDE ON THE GROWTH OF *OPHIOBOLUS GRAMINIS* IN PURE CULTURE. Jour. Agr. Research 37: 349-355, illus. 1928.



# INHERITANCE OF RESISTANCE TO BUNT, *TILLETIA TRITICI*, IN HYBRIDS OF WHITE FEDERATION AND BANNER BERKELEY WHEATS<sup>1</sup>

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## INTRODUCTION

In previous papers (1, 3, 4)<sup>3</sup> the inheritance of resistance to bunt, *Tilletia tritici*, (Bjerk.) Wint., in hybrids between susceptible varieties of wheat and the resistant varieties Martin, Hussar, and White Odessa has been reported. It was shown that Martin differs from such susceptible varieties as White Federation in one main dominant factor for resistance to this disease (1). Hussar differs from susceptible varieties in two factors, one of which is the same as the dominant factor in Martin, but the other allows bunt to develop in about half the heterozygous plants (1, 3). White Odessa has a single dominant factor for resistance which is similar in its effect to the Martin factor (4). Whether this factor is identical with the Martin factor has not been determined definitely.

The present paper is concerned with studies on the inheritance of resistance to bunt in a hybrid between susceptible White Federation and resistant Banner Berkeley.

## METHODS AND MATERIALS

The parental material and hybrid populations were grown in the field at University Farm, Davis, Calif. Conditions there favor such investigations because relatively high bunt infection can be obtained when wheat is sown in the fall. Both spring and winter varieties may be seeded at that time without any danger of winterkilling and with the assurance that both types will mature the following summer.

The seeds were thoroughly blackened with bunt spores. The inoculum was collected by W. W. Mackie in 1917 on Little Club wheat in the Montezuma Hills district of Solano County, Calif. It was propagated by Mackie on Little Club wheat in the botany garden at Berkeley, Calif. Since 1919 the writer has grown smut from this original collection on White Federation wheat at Davis. The inoculum used, therefore, has been derived from one original collection of bunt. Since Faris (6) showed that physiologic forms of bunt exist, the writer has been especially careful not to introduce new collections of bunt. The fact that the same collection of bunt has been used continuously at Davis makes it reasonably certain that the same form or mixture of forms has been employed in all these investigations. This

<sup>1</sup> Received for publication Nov. 1, 1930; issued March, 1931. Cooperative investigations of the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the division of agronomy, California Agricultural Experiment Station.

<sup>2</sup> The writer acknowledges valuable suggestions from Dr. R. E. Clausen, division of genetics, and various members of the division of agronomy, University of California, and from various members of the Office of Cereal Crops and Diseases.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 313.

is indicated also by the consistent way in which the parent wheat varieties have reacted to this inoculum.

The wheat seeds were spaced from 2 to 3 inches apart in rod rows 1 foot apart. The entire nursery was sown within three or four days, in order to avoid the effects of different temperatures and soil moistures.

At harvest the plants in each row were pulled and separated into two piles, one bunt free and the other bunted. The total number of plants and the number of bunted plants were recorded, and the percentage of bunt infection was calculated. A plant was classified as bunted if it showed any infection.

According to Tisdale et al. (9), Banner Berkeley produced an average of 1.1 per cent of bunt at Moro, Oreg., in the three years 1920 to 1922, but produced no bunt at Pullman, Wash., in 1922. The writer obtained a supply of seed from H. M. Woolman in the fall of 1921, and this variety had 3.2 per cent of bunt in 1922 at Davis, Calif. Banner Berkeley was not grown in the smut nursery again until 1927, but has been bunt free since that time. From 1922 to 1926 it was propagated in the parent nursery by growing a row each year from a single head. In 1926 one plant was crossed with White Federation and Martin wheats. The stock of seed used since that time has been propagated from that plant.

Just why Banner Berkeley produced a little bunt in 1922 and has been bunt free since 1927 under similar conditions when inoculated with bunt from the same original source is not known. A similar condition was encountered with White Odessa wheat. It is possible that the original lot of seed was not a pure line. The original collection of bunt may have consisted of two or more physiologic forms of bunt, one of which could attack Banner Berkeley slightly. The latter form could have been lost in propagating the inoculum on White Federation. Dillon-Weston (5) has shown that it is possible to isolate more than one physiologic form from a single collection of bunt. Finally, the seed of Banner Berkeley as obtained from Mr. Woolman may have been slightly contaminated with a form of bunt which could attack it. It is known now that such forms exist in Oregon.

That White Federation is very susceptible as compared with Banner Berkeley may be seen in Table 1.

TABLE 1.—*Annual percentage of bunt infection in the parent wheat varieties in four years, 1922 and 1927-1929, when grown at Davis, Calif.*

Variety	Percentage of bunted plants				
	1922	1927	1928	1929	Average
Banner Berkeley.....	3.2	0.	0.	0.	0.8
White Federation.....	58.3	66.6	68.9	78.6	68.1

### EXPERIMENTAL RESULTS

The crosses White Federation  $\times$  Banner Berkeley and Martin  $\times$  Banner Berkeley were made in 1926. The latter cross was made to determine whether the Martin factor was present in Banner Berkeley. The  $F_1$  seeds were not inoculated because of the small number available.

A part of the  $F_2$  seeds of both crosses were treated with copper carbonate to protect them from bunt infection, in order to grow a supply for  $F_3$ . Enough seeds of each cross to plant 20 rod rows were inoculated and the  $F_2$  was grown in 1928. Twenty-two additional  $F_2$  rod rows of White Federation  $\times$  Banner Berkeley were grown in 1929.

The  $F_2$  data do not permit a satisfactory Mendelian analysis, because some susceptible plants usually escape infection. Besides, resistant and heterozygous plants occasionally may become infected. They do indicate the number of factors present and also the percentage of bunted plants to be expected in  $F_3$  rows of the same genotype. Data collected in  $F_2$  are recorded in Table 2.

TABLE 2.—*Percentage of bunted plants in the parents and the  $F_2$  of the crosses named when grown in the field at University Farm, Davis, Calif.*

Parent or cross	Year grown	Number of plants		Percentage of bunted plants
		Total	Bunted	
Banner Berkeley.....	1927	151	0	0
Do.....	1928	491	0	0
Do.....	1929	532	0	0
White Federation.....	1928	309	253	81.9
Do.....	1929	492	428	87
Martin.....	1928	293	0	0
Do.....	1929	232	0	0
$F_2$ Martin $\times$ Banner Berkeley.....	1928	893	0	0
$F_2$ White Federation $\times$ Banner Berkeley.....	1928	965	230	23.8
Do.....	1929	1,034	244	23.6

There was an average of 23.6 per cent of bunted plants in the  $F_2$  of White Federation  $\times$  Banner Berkeley, which is very near the 25 per cent expected on the basis of a single dominant factor for resistance. Martin  $\times$  White Federation produced 17.2 per cent of bunted plants in  $F_2$  (1). At that time it was shown that enough susceptible plants had escaped infection to bring this figure into satisfactory agreement with the 25 per cent expected. White Odessa  $\times$  White Federation had 22.3 per cent of bunted plants in  $F_2$  (4). In the case of White Federation  $\times$  Banner Berkeley, as with the White Odessa cross, either fewer susceptible plants escaped infection or more resistant and heterozygous plants became infected. That resistant and heterozygous plants occasionally may become infected has been shown in other papers (2, 4). The  $F_2$  data, then, indicate that Banner Berkeley differs from White Federation in a single dominant factor for resistance to bunt, and that heterozygous  $F_3$  rows should contain an average of about 24 per cent of bunted plants.

$F_2$  of Martin  $\times$  Banner Berkeley was free from bunt, indicating that Banner Berkeley has the same factor for resistance as Martin. This point is confirmed by  $F_3$  data, which will be presented later.

In  $F_3$ , 301 rod rows were grown from 301  $F_2$  plants which had been protected from bunt infection by seed treatment. Obviously the classification of  $F_2$  plants on the basis of the behavior of their progeny in  $F_3$  rows is more reliable than classification in  $F_2$ .  $F_3$  rows contained from 30 to 60 plants.  $F_3$  data are recorded in Table 3.

TABLE 3.—Distribution of the parent and the  $F_2$  rows of the crosses named into 5 per cent classes for bunt infection, when grown at Davis, Calif., in 1929

Parent or cross	Distribution of rows by percentage classes of bunt infection																				Total number of rows	
	0	1-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	65-70	70-75	75-80	80-85	85-90	90-95		95-100
White Federation																						10
Martin		5																4	3	2	1	5
Banner Berkeley	12																					12
White Federation×Banner Berkeley	57	10	4	15	32	59	29	19	5	3	1	0	2	1	0	3	12	13	10	16	10	301
Martin×Banner Berkeley	100																					100

The rows in the 0-5 per cent class were separated into those with no bunted plants and those with 1 to 5 per cent of bunted plants because of the special interest in the former. The nature of the distribution may be seen more readily in Figure 1.

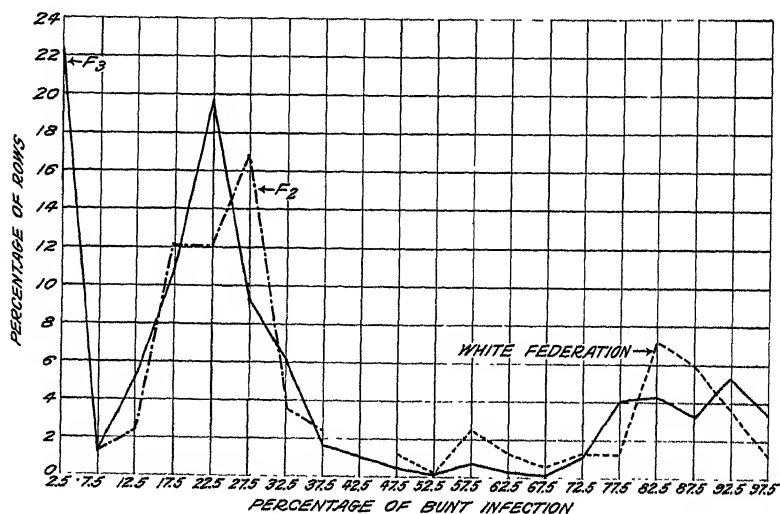


FIGURE 1.—Distribution of  $F_2$  and  $F_3$  rows of the cross White Federation  $\times$  Banner Berkeley and rows of the White Federation parent into 5 per cent classes for bunt infection. (The solid line represents 301  $F_3$  rows, the dot-and-dash line 42  $F_2$  rows, and the broken line 42 rows of White Federation)

The numbers of rows under the three modes agree satisfactorily with the 1:2:1 ratio. There were 69 resistant rows where 75.25 were expected, 165 heterozygous rows where 150.5 were expected, and 67 susceptible rows where 75.25 were expected. The minima should not be thought of as marking with absolute accuracy the divisions between phenotypes. However, the first minimum in the crosses with Martin, White Odessa, and Banner Berkeley fell at 7.5 per cent of bunt. In the same crosses the second minimum fell very near 50 per cent. The numbers of rows in this region are so few that any point from 40 to 60 would not change materially the numbers of rows under the adjacent modes.

The 69 resistant rows were made up of 57 which were completely resistant and 12 which had from 1 to 7.5 per cent of bunted plants.

Data have been published (2) which show that this percentage of bunt may be due to modifying factors and that these rows do belong in the resistant group.

There were 67 susceptible rows, which contained from 52.5 to 97.5 per cent of bunted plants. The 10 rows of White Federation which were grown as checks among the 301  $F_3$  rows of White Federation  $\times$  Banner Berkeley had from 82.5 to 97.5 per cent of bunt. These did not approach the lower limits of bunt found in susceptible  $F_3$  rows, but the number of parent rows was small. In the entire smut nursery there were 42 rows of White Federation, which had from 47.5 to 97.5 per cent of bunted plants. These are shown in Figure 1 as a broken line. The distribution of the 42 rows of White Federation agrees very well with the distribution of susceptible  $F_3$  rows. The writer usually plants in paired rows. The two rows commonly differ in bunt infection by 5 to 10 per cent. In one case adjacent rows of the above lot differed by 30 per cent, indicating that environmental conditions may change radically from row to row.

There were 165 heterozygous rows with an average of 24.1 per cent of bunted plants. Twenty  $F_2$  rows produced an average of 23.8 per cent of bunted plants in 1928, and 22  $F_2$  rows averaged 23.6 per cent in 1929. The 42  $F_2$  rows are plotted with a dot-and-dash line over the heterozygous group of  $F_3$  rows in Figure 1. The two curves agree very well in essential features.

TABLE 4.—Distribution of  $F_4$  rows from certain  $F_3$  rows of the cross White Odessa  $\times$  White Federation into 5 per cent classes for bunt infection, when grown at Davis, Calif., in 1929

Distribution of rows by percentage classes of bunt in- fection	F <sub>4</sub> rows from E <sub>3</sub> row No. 1092 with 13.1 per cent of bunt		F <sub>4</sub> rows from F <sub>3</sub> row No. 1347 with 14.6 per cent of bunt		F <sub>4</sub> rows from F <sub>3</sub> row No. 1349 with 31.7 per cent of bunt		F <sub>4</sub> rows from F <sub>3</sub> row No. 1360 with 36.7 per cent of bunt	
	Classes	Modes	Classes	Modes	Classes	Modes	Classes	Modes
0.....	11	15	13	14	11	12	2	6
1-5.....	3		2		1		3	
5-10.....	2		5		1		1	
10-15.....	10	37	9	28	3	26	1	17
15-20.....	9		2		8		1	
20-25.....	8		7		7		1	
25-30.....	3		4		3		2	
30-35.....	4				2		3	
35-40.....	2				1		6	
40-45.....					1		2	
45-50.....	1		1				1	
50-55.....	1	9		7		15		6
55-60.....	1				1			
60-65.....	1							
65-70.....	2				1			
70-75.....					1			
75-80.....								
80-85.....					1			
Total rows.....	61		49		53		29	
P.....	0.122		0.234		0.801		0.654	

<sup>a</sup> Includes 3 plants that were completely smutted in  $F_3$  and therefore produced no good seed.

<sup>b</sup> Includes 6 plants that were completely smutted in  $F_3$  and therefore produced no good seed.

<sup>c</sup> Includes 5 plants that were completely smutted in  $F_3$  and therefore produced no good seed.

<sup>d</sup> Includes 11 plants that were completely smutted in  $F_3$  and therefore produced no good seed.

Obviously it is not practicable to check the accuracy of the classification of very many  $F_3$  rows by growing the  $F_4$ . Four  $F_3$  rows of the heterozygous group from White Odessa  $\times$  White Federation (4) now



have been checked in  $F_4$ . Two rows were selected from near the lower limits and two rows from near the upper limits for bunt infection in the heterozygous group. These rows had, respectively, 13.1, 14.6, 31.7, and 36.7 per cent of bunted plants. The extreme limits of the heterozygous group were avoided, because there is no reason to believe that there is not some overlapping. It therefore would have been desirable to check a larger number of  $F_3$  rows, which was not practicable. That the four rows described belong to the heterozygous group may be seen from Table 4.

If the plants that were completely smutted in  $F_3$  are classed as susceptible and are added to the  $F_4$  rows that are classed as susceptible, the agreement with the 1:2:1 ratio in all four families is satisfactory. Table III by Fisher (7) was used in calculating the  $P$  values. These four  $F_3$  rows were properly classified as heterozygous.

The classification of  $F_2$  plants, determined on the basis of the percentages of bunted plants in  $F_3$  rows, shows that Banner Berkeley differs from White Federation in one main dominant factor for resistance to bunt. That this factor is identical with the factor in Martin is evidenced by the fact that there was no bunt in the  $F_2$  or 100  $F_3$  rows of Martin  $\times$  Banner Berkeley.

#### DISCUSSION AND SUMMARY

The literature pertinent to the inheritance of resistance to bunt has been reviewed and discussed in earlier publications and will not be repeated here.

The writer has presented data (1) which show that the Martin variety differs from such susceptible wheats as White Federation in one main dominant factor for resistance to bunt. Hussar wheat (1, 3) differs from such susceptible varieties in two factors for resistance to this disease. One is the same as the factor present in Martin, but the other allows bunt to develop on about half of the heterozygous plants. Martin may be represented by  $MMhh$  and Hussar by  $MMHH$ . White Odessa wheat (4) has a single factor for resistance to bunt which is similar in its effect to the factor present in Martin. Whether or not it is identical with the Martin factor is not known at present. The data now available suggest that they are identical and that White Odessa should be designated as  $MMhh$ . Data presented in this paper show that Banner Berkeley differs from White Federation in one main factor for resistance to bunt. This factor is identical with the one present in Martin, therefore Banner Berkeley is of the  $MMhh$  constitution.

The bunt used in all these experiments is the one designated as physiologic race III of *Tilletia tritici* by Reed (8). There may be other factors for resistance to bunt in Martin, Hussar, White Odessa, and Banner Berkeley which would have become apparent in the presence of other physiologic forms of bunt. If there are no other factors present, Martin ( $MMhh$ ), Banner Berkeley ( $MMhh$ ), and White Odessa, if the  $MMhh$  designation is correct, should react the same to all physiologic forms of bunt. Furthermore, Hussar ( $MMHH$ ) should not be susceptible to any forms to which the Martin group is resistant. However, Hussar should be resistant to some forms which attack Martin, because of the  $H$  factor. There are not enough data available at present to verify this point.

The study of physiologic forms of bunt has been complicated somewhat because monosporic inoculations have not been possible with this disease. The situation will be improved wherever it is possible to harbor a particular physiologic form of bunt on a variety that is resistant to all other forms that are being investigated. That field collections of bunt may not be pure physiologic forms has been shown by Dillon-Weston (5). He was able to isolate a very virulent form from a relatively nonvirulent collection of inoculum when used on Sherman wheat. Undoubtedly he was dealing with a mixture of forms in which the virulent form was present in relatively small amounts.

In breeding bunt-resistant varieties of wheat it is immaterial, as far as bunt resistance is concerned, whether Martin or Banner Berkeley is used for the resistant parent, because they both have the *MMhh* constitution for resistance.

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## A GENETIC STUDY OF WHEAT×RYE HYBRIDS AND BACK CROSSES<sup>1</sup>

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### INTRODUCTION

A number of present-day wheat breeders are back crossing wheat × rye hybrids as a possible means of improving winter wheat. Although bread wheats are superior in quality to rye, some rye varieties possess certain characters, such as winter hardiness, which would be very valuable if they could be transferred to wheat. A limited degree of success in transferring rye characters to wheat has been achieved, but the manner of transmission is not definitely known. The high degree of sterility characteristic of wide crosses such as that between wheat and rye has not been overcome completely in the new strains possessing rye characters. The main objects of the experiments here reported were to obtain further information on the manner in which rye characters may be transferred to wheat and to study the degree and causes of sterility and the relationship of characters in successive generations of hybrids.

### REVIEW OF LITERATURE

There is a considerable body of literature on experiments with wheat × rye hybrids. A fairly complete review of such experiments has been made by Bleier (2)<sup>3</sup> and others. Only those references which have a bearing on the present paper will be cited here.

The wheat × rye cross<sup>4</sup> usually is not difficult to accomplish, but until recently the reciprocal cross has been considered impossible. Many workers report only a low percentage of kernels from wheat × rye crosses, while others report a high percentage. Backhouse (1), using a Chinese wheat supplied by Biffen, reported percentages up to 80. Thompson (16), utilizing the same wheat, stated that it was not difficult to get 90 per cent of crossed kernels in P<sub>1</sub>. Nina Meister and Tjumjakoff (13) reported up to 60 per cent. Leighty and Sando (10), also crossing a Chinese variety with rye, obtained a 90.5 per cent set of seed. The variety of Chinese wheat used by Leighty and Sando has a clavate spike and is red kerneled, while the one used by

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<sup>2</sup> The writer wishes to express appreciation to R. E. Clausen, professor of genetics, University of California, for his helpful suggestions in this experiment, and to J. Allen Clark, of the Office of Cereal Crops and Diseases, for suggestions as to crosses and parental material. G. A. Wiebe, assistant agronomist in charge of the cooperative cereal-agronomy investigations at University Farm, Davis, Calif., assisted materially in providing facilities for growing and harvesting F<sub>4</sub> material at Davis in 1929.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 338.

<sup>4</sup> In the designation of a cross the female parent will appear first throughout this paper.

Backhouse and Thompson has a fusiform spike with white kernels. Poor results from crossing no doubt may be due in part to variation in compatibility to rye in different varieties of wheat. Firbas (4) crossed a number of varieties of wheat with several varieties of rye and found distinct differences among the wheats.

In 1922 Gaines and Stevenson (6) reported maternal inheritance in a rye  $\times$  wheat cross. The plants were rye-like and had only a moderately high degree of sterility. The chief criterion of hybridity employed by these investigators was susceptibility to disease, which was not observed in the rye parent.

Nina Meister and Tjumjakoff (13), in 1927, succeeded in making rye  $\times$  wheat crosses. From 3,894 crossed rye florets, 96 kernels, or 2.5 per cent of the total number, were obtained. The hybrids were identical with the usual  $F_1$  wheat-rye crosses. The authors attributed their success to the use of a specially constructed greenhouse where the plants were grown. Maintenance of proper humidity for the germinating wheat pollen was considered an important factor.

Wheat normally is self-fertilized and rye cross-fertilized. The earlier breeders usually obtained some fertility in  $F_1$  crosses. Jesenko (7) appears to have been the first to make a definite contribution to the knowledge of sterility relationships in the hybrids. The  $F_1$  hybrids do not dehisce and shed their pollen, as both wheat and rye normally do. Jesenko opened the anthers artificially and applied the pollen to the florets of the hybrid. One kernel was obtained, which he later concluded must have resulted from chance pollination by wheat. The following year about 3,000 florets of the  $F_1$  were again selfed. Not a single kernel was obtained. Since the pollen of wheat as well as of rye may be carried about by winds at blossoming time, he concluded that the progeny reported by other workers must have been due to chance fertilization by such pollen.

Meister (12) isolated 220 heads of natural wheat  $\times$  rye hybrids at blossoming time. All were entirely sterile.

Leighty and Taylor (11) bagged 176 individual heads and compared their fertility with that of 309 unbagged heads. The former were completely sterile, while in the latter a fertility of 0.66 percent was observed.

Since  $F_1$  hybrids were thus definitely proved to be sterile, the chance pollinations found largely determined the direction of further investigation of wheat  $\times$  rye hybrids. Jesenko (8) compared both the wheat and the rye parent in back crossing on the hybrid. With the wheat back crosses he obtained from three to six kernels per 1,000 florets pollinated. In 1912 one kernel was obtained from 4,800  $F_1$  flowers back crossed with rye, the plant produced being rye-like.

The main effort of plant breeders working with hybrids of wheat and rye has been directed to the improvement of wheat. For this purpose hybrids from chance pollinations and from artificial back crosses with wheat have been used. Efforts are being made to fix desirable rye characters in true-breeding back-crossed strains. Meister (12), in 1921, reported segregation for frost resistance in winter wheat  $\times$  winter rye crosses, and in a verbal statement in 1927 he reported true-breeding strains of winter wheats of increased hardiness. Leighty and Taylor (11) reported the occurrence of three apparently true-breeding "hairy-neck" strains in the  $F_2$  progeny of an  $F_1$  wheat  $\times$  rye hybrid.

## PARENT VARIETIES

The original crosses used in these experiments were made between seven varieties of wheat and two of rye. Experimental data collected in the later generations were confined to three crosses involving Rosen and Dakold ryes and Kanred and Hybrid 128 wheats. The rye varieties were obtained from the Dickinson substation, Dickinson, N. Dak. The wheats were pure lines obtained from varieties previously grown by the writer in experiments at University Farm, Davis, Calif.

## ROSEN RYE

Rosen rye (*Secale cereale* L.), C. I.<sup>5</sup> 195, was obtained originally from Russia in 1908 by the Michigan Agricultural Experiment Station as an unnamed variety (14). It is a slightly hardy winter variety with comparatively short, faintly purple to purple, stiff straw, broad leaves, and broad, flat, dense heads. The peduncle immediately below the head usually has a dense coat of hair or pubescence for a distance of about 2 inches. The term "hairy neck," first applied to this character by Leighty and Taylor (11), will be used in the present paper. The kernels are normally 7.5 mm. long but vary in different seasons. In the experiments here reported, the kernel color usually was pale red or yellowish; but gray and green kernels nearly always were present. Rosen rye is slightly more winter hardy than Kanred wheat.

## DAKOLD RYE

Dakold rye (*Secale cereale* L.), N. Dak. No. 959, is an early-maturing winter variety developed at the North Dakota Agricultural Experiment Station (15). In early growth it is more prostrate than Rosen and has narrower leaves. The stems are slender, medium tall, and light purple to purple in color, with strong hairy necks. The spikes are slender, and the kernels are dark reddish yellow or greenish, and small, averaging about 7 mm. in length. Dakold is known particularly for its winter hardiness.

## KANRED WHEAT

Kanred wheat (*Triticum vulgare* Vill.), C. I. 5146, has a distinctly prostrate winter habit with fine dark-green leaves, is midseason in maturity, and has white, weak, mid-tall stems with glabrous peduncles. The spikes are awned, fusiform, mid dense; the glumes glabrous, white; and the beaks from 3 to 25 mm. long. The kernels are red and vary from 6.5 to 6.9 mm. in length. Kanred is moderately winter hardy.

## HYBRID 128 WHEAT

Hybrid 128 wheat (*Triticum compactum* Host.) was originated at the Washington Agricultural Experiment Station from a cross between Jones Winter Fife (*T. vulgare* Vill.) and Little Club (*T. compactum* Host.) (3). It is a uniform, constant, true-breeding, winter-habit, mid-season variety with white, stiff, strong, mid-tall stems and glabrous peduncles. The spikes are dense, erect, with short-tip awns, and the glumes are glabrous, white, with beaks 0.5 mm. long. The kernels are white and vary from 5.6 to 5.8 mm. in length. Hybrid 128 is a tender winter wheat.

<sup>5</sup> C. I. denotes accession number of Office of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

## OTHER VARIETIES

A number of other wheats and wheat allies were crossed with rye in the initial stages of the experiment. No description of these will be given. They included Red Winter spelt (*Triticum spelta* L.), C. I. 1772; Peliss (*T. durum* Desf.), C. I. 1584; Clackamas (*T. durum* Desf.), C. I. 6241; Black Winter emmer (*T. dicoccum* Schrk.), C. I. 2337; and Polish wheat (*T. polonicum* L.), C. I. 3001.

## EXPERIMENTAL METHODS AND DATA

All the hybrid and parental material, except a limited number of plants grown at Berkeley for cytologic study in 1929, was grown in the cereal nursery at University Farm, Davis, Calif. As the parental varieties were of winter habit, only one generation was grown each year. All sowings were made in the fall, mostly in November. Seed from the  $F_1$  and the  $P_1$  back-crossed generations was spaced 4 to



FIGURE 1.—Bagged second back-cross second-generation plants of Hybrid 128 wheat  $\times$  Rosen rye, Davis, Calif., 1928

6 inches apart in rows 1 foot apart, and the segregating generations (1928–29) 2 inches apart in the rows.

In making crosses and back crosses common white paper or glassine bags were used to protect emasculated and pollinated heads from chance pollination. All plants from back-crossed kernels and two selfed generations (in the period from 1925–1928) were covered with 30-pound manila paper bags to prevent chance pollination. (Fig. 1.) Each plant was tied to a stake. Development appeared to proceed in a fairly normal manner in covered plants, although there was some reduction in vigor and fertility.

At harvest the plants were pulled and taken to the laboratory for description and study. The characters studied in most of these plants include color of kernel (in crosses involving Hybrid 128), hairy neck, length of kernel, fertility, development of kernel, height of culm, and length of spike. Fertility was determined as percentage calculated from the number of kernels produced in a given number of florets, or as the average number of kernels per spikelet, usually

calculated from two spikes per plant. To obtain the average length of kernel, 10 average kernels from each plant were laid end to end in a narrow crease in a flat board and measured.

#### FIRST-GENERATION WHEAT × RYE CROSSES IN 1924

Hybrid 128 and Kanred wheats, Red Winter spelt, Clackamas and Peliss wheats, Black Winter emmer, and Polish wheat were crossed with Rosen and Dakold ryes. (Table 1.) Crosses were obtained readily with varieties of the 42-chromosome wheats. None was obtained with Black Winter emmer and very few with Polish, Peliss, and Clackamas wheats. Crossed kernels were more or less shriveled or misshapen in many cases. Kernels from Hybrid 128 and Kanred ranged from fairly plump to shriveled. In Hybrid 128 there were a number of very small kernels.

TABLE 1.—*Wheat × rye crosses made at University Farm, Davis, Calif., 1924 and 1926*

Year and cross	Chromosome group of wheat parent	Spikes used	Florets crossed	Kernels obtained	Florets setting seed
1924					
Red Winter × Rosen	42	4	96	a 17	17.7
Red Winter × Dakold	42	4	96	a 24	25.0
Hybrid 128 × Rosen	42	4	96	b 70	72.9
Hybrid 128 × Dakold	42	4	96	b 81	84.4
Kanred × Rosen	42	4	96	c 30	31.3
Kanred × Dakold	42	4	96	c 57	59.4
Peliss × Rosen	28	4	96	c 2	2.1
Peliss × Dakold	28	4	96	c 2	2.1
Black Winter × Rosen	28	4	96	0	0
Black Winter × Dakold	28	4	96	0	0
Polish × Rosen	28	3	72	0	0
Polish × Dakold	28	3	72	a 2	2.8
Clackamas × Rosen	28	3	72	c 2	2.8
Clackamas × Dakold	28	3	72	c 8	11.1
1926					
Hybrid 128 × Rosen	42	2	54	23	42.7
Red Winter × Rosen	42	2	48	3	6.3
Kanred × Rosen	42	2	50	3	6

a Fairly well developed.

b Fairly well developed but small.

c Badly shriveled.

Observations and measurements were made on height of plant, type of spike, length of main spike, hairy neck, length of beak, and color of straw. Most of these characters were intermediate or inclined to one or the other parent. Height of plant was inclined strongly to that of the rye parent. The spike in the  $F_1$  hybrid of crosses with Hybrid 128 wheat is a long-club type. (Fig. 2, B.) The length of spike in Red Winter spelt × Dakold rye exceeded that of the parents. Hairy neck was heavy on most plants, but it was absent on a few. This variation in  $F_1$  probably is due to the fact that a small percentage of Rosen plants were glabrous. Length of beak of the outer glume, considered a very constant character in wheat, was distinctly wheatlike. At Davis, the  $F_1$  hybrid and the rye parents both had purple straw, and the wheat parents had white straw. The coleoptiles and leaf sheaths of  $F_1$  seedlings were reddish purple like those of the rye parents. (Table 2.)



At blossoming time the glumes opened, permitting the anthers to extrude. The glumes have a tendency to remain open for a consid-



FIGURE 2.—Parents and  $F_1$  hybrid of Hybrid 128 wheat  $\times$  Rosen rye. A, Rosen; B,  $F_1$  hybrid; C, Hybrid 128. (Two-thirds natural size)

erable time. No anthers were observed to shed their pollen. On several occasions anthers were collected for pollen examination. Only a few abnormal pollen grains of variable size were found.

TABLE 2.—Description of  $F_1$  plant characters in wheat × rye crosses, 1925

Cross or parent	Plants	Height of plants	Type of spike	Length of main spike	Hairy neck	Length of beak	Color of straw
	Number	Inches		Cm	Degree	Mm.	
Red Winter × Dakold.	2	53-60	Lax, speltlike.	14 -16	Heavy	0 5-1	Purple
Hybrid 128 × Dakold.	6	53-54	Compact, long club.	7.5- 9	3 light at top, 3 none	5- 1	Do.
Hybrid 128 × Rosen.	5	50-60	.....do.	6 - 7	4 heavy; 1 light.	5- 1	Do.
Kanred × Rosen.	3	55-58	Lax.	12 -13	1 medium heavy, 2 none	5 -10	Do.
Kanred × Dakold.	3	56-61	.....do.	14 -15 5	Medium heavy.	5 -15	Do.
Hybrid 128.	10	43-52	Compact, club.	4 5- 5.5	None.	5	White
Kanred.	10	47-52	Lax.	8.5-10	.....do.	3 - 8	Do.
Rosen.	5	58-65	Long, lax.	12 -18	2 medium heavy, 3 light.	.....	Purple.

# EFFECT OF BAGGING ON FERTILITY OF $F_1$ HYBRIDS AND WHEAT PARENTS

The effect of isolation on the fertility of the  $F_1$  plants was studied. Seventy-seven heads containing 4,245 florets were covered individually with glassine bags a short time before flowering. All heads were completely sterile. Nineteen heads on five crosses not covered yielded kernels only in the two crosses involving Hybrid 128. From 11  $F_1$  heads, containing 1,334 florets of the crosses with Hybrid 128, 8 kernels were obtained, equaling 0.6 per cent, or 6 kernels per 1,000 flowers. (Table 3.)

TABLE 3.—Relative fertility of  $F_1$  wheat × rye hybrids when bagged and when not bagged, 1925

Cross	Bagged				Not bagged			
	Heads	Florets	Kernels obtained	Fertility	Heads	Florets	Kernels obtained	Fertility
	Number	Number	Number	Per cent	Number	Number	Number	Per cent
Red Winter × Dakold.	13	743	0	0	2	170	0	0
Hybrid 128 × Dakold.	18	950	0	0	6	694	6	.86
Hybrid 128 × Rosen.	15	866	0	0	5	640	2	.31
Kanred × Rosen.	14	746	0	0	3	328	0	0
Kanred × Dakold.	17	940	0	0	3	360	0	0

A number of heads of Dakold and Rosen rye also were covered with glassine bags. The fertility of Dakold was 1.6 per cent and that of Rosen 5.4 per cent. Although a high degree of self-sterility is normal in rye, the high temperature and low humidity during the blooming period no doubt increased the sterility in the covered heads.

The effect of bagging on fertility in Hybrid 128 and Kanred wheats was studied in 1928. The average number of kernels per spikelet in bagged Hybrid 128 was 1.79, and in unbagged, 2.18; in bagged Kanred, 1.64, and in unbagged, 1.92. The average reduction in length of kernel from bagging in Hybrid 128 was 0.2 mm., and in Kanred, 0.3 mm. Bagging increased the number of rudimentary spikelets 2.7 per cent in Hybrid 128, but decreased them 1.9 per cent in Kanred. (Not shown in Table 4.) Bagging individual plants reduced the fertility 17.9 per cent in Hybrid 128 and 15.0 per cent in Kanred. (Table 4.)

TABLE 4.—*Relative fertility of wheat varieties when bagged and when not bagged, 1928*

Variety	Plants	Heads	Spikelets	Total kernels	Average kernels per spikelet	Average length of kernel	Reduction in fertility
	Number	Number	Number	Number	Number	Mm.	Per cent
Hybrid 128 <sup>a</sup> .....	10	19	402	719	1.79	5.5	17.9
Hybrid 128 <sup>b</sup> .....	10	20	444	969	2.18	5.7	.....
Kanred <sup>a</sup> .....	10	20	358	587	1.64	6.3	15.0
Kanred <sup>b</sup> .....	10	20	347	667	1.92	6.6	.....

<sup>a</sup> Individual plants bagged.<sup>b</sup> Not bagged.

In Rosen rye a higher degree of sterility occurred in individually bagged heads than in individually bagged plants.

## THE FIRST BACK CROSS

## FIRST-GENERATION PLANTS, 1925

Since the wheat×rye  $F_1$  generation is completely self-sterile, it is necessary to back-cross with pollen from one parent or the other in order to obtain fertilization. Wheat pollen only was used in these experiments. For all back crosses in 1925, 49 heads, containing 2,228 florets, were pollinated, and 39 kernels were obtained. Fertility percentages were 1.2 and 1.3 in Hybrid 128 back crosses, and 1.5 and 2.6 in Kanred back crosses. (Table 5.) From the open-pollinated  $F_1$  Hybrid 128 wheat×rye plants, 0.86 and 0.31 per cent kernels were obtained. (Table 3.)

TABLE 5.—*Number of kernels obtained in back-crossing (wheat × rye) × wheat, 1925*

Cross	Heads crossed	Florets pollinated	Kernels obtained	Fertile florets	Average kernels per spikelet
	Number	Number	Number	Per cent	Number
(Hybrid 128×Dakold)×Hybrid 128.....	12	434	5	1.2	0.024
(Hybrid 128×Rosen)×Hybrid 128.....	10	452	6	1.3	.026
(Kanred×Dakold)×Kanred.....	12	606	9	1.5	.030
(Kanred×Rosen)×Kanred.....	15	736	19	2.6	.052

FIRST-GENERATION PLANTS ( $WR_1W_1$ ),<sup>6</sup> 1926

The characters of the back-crossed progeny of three hybrids, Hybrid 128×Rosen, Kanred×Dakold, and Kanred×Rosen, obtained by back crossing on the  $F_1$  with the wheat parent, were studied.

<sup>6</sup> The designation  $WR_1W_1$  used throughout this paper denotes that the  $F_1$  of the wheat×rye cross (written as  $WR_1$ ) has been back crossed with the wheat parent  $W$ , resulting in the plant or series of plants in question. The formula without the subscript, or generation numeral, as  $WR_1W$ , refers to the crossed kernels, or  $P_1$ , of the back cross in question. The generation of the back cross is designated by the usual subscript numeral, as  $WR_1W_1$ ,  $WR_1W_2$ , etc.  $WR_1W_2$  indicates that the plant  $WR_1W_1$  has been selfed, producing a second generation. Likewise,  $WR_1W_1W_1$  indicates that the original  $F_1$  hybrid has been back crossed with wheat and the resulting hybrid again back crossed with the wheat parent, producing the plant thus designated. When this plant is selfed the resulting plant is indicated by  $WR_1W_1W_2$ , and later selfed generations by the usual subscript numerals 3, 4, etc. In crosses involving unequal chromosomes, or chromosomes which do not pair, plants from the back cross on  $F_1$  usually vary somewhat in type, unlike the  $F_1$  plants of ordinary crosses, on account of variability in constitution of the gametes of the hybrid.

Twenty-five back-crossed plants in addition to parents were grown in 1926. These plants too were more wheatlike than those in the  $F_1$

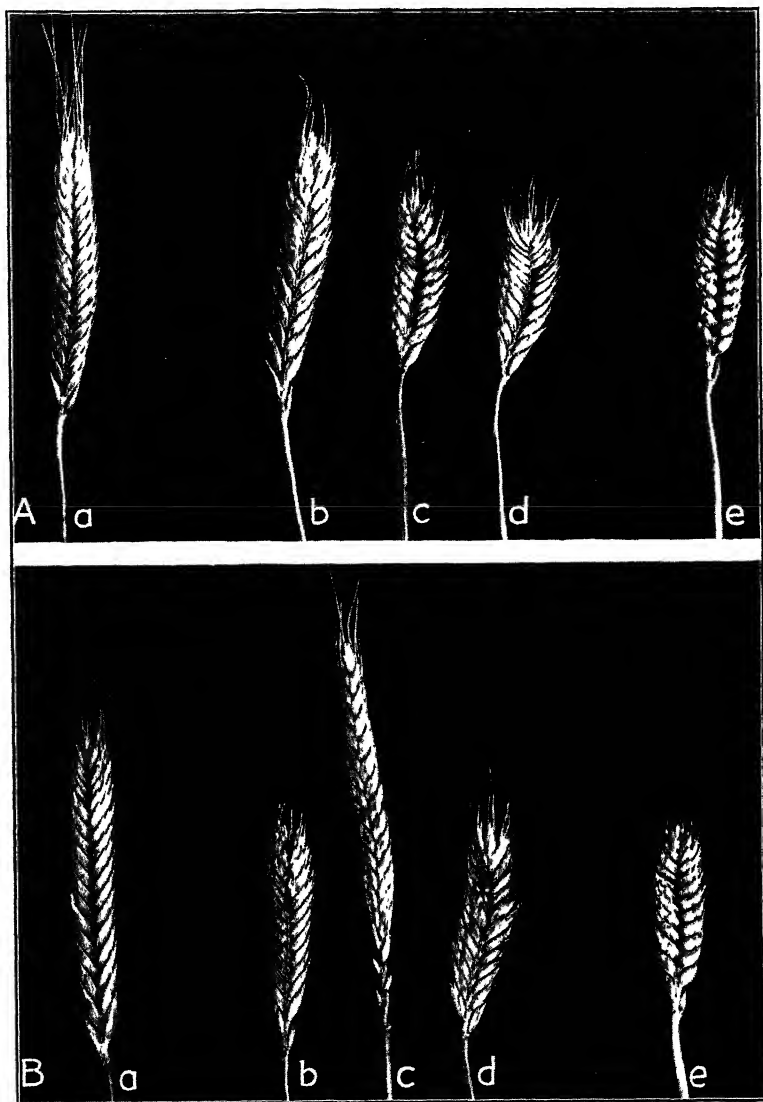


FIGURE 3.—Back-crossed first-generation hybrids and parents of Hybrid 128 wheat × rye. A, Hybrid 128 wheat × Rosen rye; B, Hybrid 128 wheat × Dakold rye; A, a, and B, a,  $F_1$  hybrid parents; A, e, and B, e, Hybrid 128; A, b, c, d, and B, b, c, d,  $WR_1W_1$  hybrid heads. Note variation in  $WR_1W_1$  heads due to variable constitution of ovules

generation, but rye characters persisted. Segregation into several different types occurred, which showed that there must have been differences in constitution of the  $F_1$  gametes. (Fig. 3 and Table 6.)

TABLE 6.—*Characters of first back-cross first-generation (wheat × rye) × wheat hybrids, 1926*

Cross, parent, and cross No.	Plant characters					Kernel characters		
	Length of main spike	Hairy neck	Fertility			Color	Average length	Development
			Flor-ets	Kernels obtained				
Hybrid 128 × Rosen: <sup>a, b</sup>	<i>Cm.</i>	<i>Degree</i>	<i>Number</i>	<i>Number</i>	<i>Per cent</i>		<i>Mm.</i>	
42A1.....	8	Light.....	88	10	11.4	Red.....	6.9	Slightly shriveled.
42B1.....	5.5	None.....	84	0				
42C1.....	5.5	do.....	94	2	2.1	Red.....	6	One kernel shriveled.
Kanred × Dakold: <sup>a, b</sup>								
43A1.....	11	Medium heavy.....	74	0				
43B1.....	6	Light at top.....	50	0				
43B2.....	9	do.....	62	0				
43C1.....	9.5	do.....	68	13	19.1	Red pale.....	7.1	Medium plump.
43D1.....	9.5	None.....	76	0				
43D2.....	8	Light.....	34	0				
43D3.....	8.5	Medium heavy.....		0				
Kanred × Rosen: <sup>a, b</sup>								
44A1.....		None.....	34	0				
44B1.....		do.....	56	3	5.4	Red pale.....	5.7	Plump.
44B2.....		do.....		0				
44C1.....		do.....	72	0				
44C2.....		do.....	62	0				
44D1.....	14.5	Light.....	82	3	3.7	Red.....	7.3	Badly shriveled.
44E1.....		Medium heavy.....		0				
44F1.....		None.....		0				
44F2.....		do.....		0				
44G1 <sup>c</sup> .....	16	Very light.....	98	4	4.1	Red.....	6.8	Slightly shriveled.
44A2 <sup>c</sup> .....	15	Light.....	84	2	2.4	do.....	7	Badly shriveled.
44C3 <sup>c</sup> .....	11	None.....	78	4	5.1	Red pale.....	7.5	Slightly shriveled.
44D2 <sup>c</sup> .....	11	Medium heavy.....	78	0				
44E2 <sup>c</sup> .....		None.....	64	5	7.8	Red pale.....	7	Do.
44F3 <sup>c</sup> .....	12	do.....	76	12	15.8	do.....	8.2	Medium plump.
Dakold.....	11	Very heavy.....	128	9	7	Red.....	6.4	Badly shriveled.
Rosen.....	15	Heavy.....	160	55	34.4	Gray, few light red.	6.5	Medium but shorter than normal.
Kanred <sup>c</sup> .....	10	None.....	283	672	2.37	Red.....	6.9	Plump.
Hybrid 128 <sup>c</sup> .....	5	do.....	71	124	1.75	White.....	5.1	Badly shriveled.

<sup>a</sup> Parent wheat varieties were used in back crossing on F<sub>1</sub> in all cases. Heads of (Hybrid 128 × Rosen) × Hybrid 128 long-club type in plant 42A1; others short club. All heads lay in Kanred × rye × Kanred crosses.

<sup>b</sup> Plants bagged.

<sup>c</sup> Plants not bagged.

<sup>d</sup> Average of 10 plants.

<sup>e</sup> Average of 2 plants.

<sup>f</sup> Number of spikelets.

<sup>g</sup> Kernels per spikelet.

All back crosses segregated for hairy neck. The expression of hairy neck varied from very light at the top of the peduncle to medium and heavy. Of 3 Hybrid 128 wheat × Rosen rye back-crossed plants, 1 had light hairy neck and 2 had none; of the Kanred wheat × Dakold rye back-crossed plants, 6 had medium and light hairy neck and 1 had none; and of the Kanred wheat × Rosen rye back-crossed plants, 5 had medium and light hairy neck and 10 had none. Two plants of the Hybrid 128 wheat × Rosen rye back cross produced red kernels; the third plant was sterile.

The type of head varied mainly in size and length (column 5, Table 2), but almost exact parental duplicates were represented. Similarly, length of kernel varied from shorter than that of the short-kernelled parent to longer than that of Rosen. Fifteen of the selfed plants were sterile. The fertility of the remainder was variable up to about 19 per cent. Seed from 3 of the 10 plants producing kernels was well developed, while that from the remainder was more or less badly shriveled.

## THE SECOND BACK CROSS

## RELATIVE FERTILITY FROM CROSSING AND SELFING

In order to get strains of wheatlike material possessing rye characters as soon as possible—for example, hairy neck on a wheatlike plant—the back-cross progeny plants were again back crossed with their respective wheat parents. Seventeen plants were back crossed. Data on the fertility of these plants, when back crossed the second time and when selfed, are given in Table 7. In most cases a higher set of seed was obtained from back-crossed plants than from selfed plants. Plump kernels were obtained in several plants. On back crossing, a lower set of seed, with more shriveled kernels, was obtained from the apparently self-sterile plants than from the partly self-fertile plants. Fifty-eight heads containing 1,779 florets were emasculated and pollinated, and 323 kernels were obtained. The average fertility was 18.2 per cent.

TABLE 7.—*Relative fertility of selfed and back-crossed first-generation (wheat × rye) × wheat hybrids, 1926*

Cross and No.	Parent plants back crossed					Parent plants selfed (bagged)				
	Heads crossed	Florets pollinated	Kernels			Heads examined	Florets	Kernels		
			Obtained	Condition	Seed set			Obtained	Condition	Seed set
Hybrid 128 × Rosen	No.	No.	No.		P. ct.	No.	No.	No.		P. ct.
42A1 × Hybrid 128	3	100	38	Plump and shriveled.	38.0	2	88	10	Slightly shriveled.	11.4
42B1 × Hybrid 128	5	144	4	Badly shriveled.	2.8	2	84	0		
42C1 × Hybrid 128	7	260	12	Plump.	4.6	2	94	2	1 shriveled; 1 plump.	2.1
Kanred × Dakold:										
43A1 × Kanred	3	96	3	do	3.1	2	74	0		
43B1 × Kanred	3	94	27	do	28.8	2	60	0		
43B2 × Kanred	5	129	20	Plump, some shriveled.	15.5	2	62	0		
43C1 × Kanred	2	52	28	Plump.	53.8	2	68	13	Medium plump.	19.1
43D1 × Kanred	3	98	15	Shriveled.	15.3	2	76	0		
43D2 × Kanred	2	56	2	Plump.	3.6	1	34	0		
43D3 × Kanred	5	159	17	do	10.7			0		
Kanred × Rosen:										
44A1 × Kanred	2	74	7	Medium plump.	9.5	1	34	0		
44B1 × Kanred	3	92	73	Plump.	79.4	2	56	3	Shriveled.	5.4
44B2 × Kanred	3	78	4	Medium plump.	5.1			0		
44C1 × Kanred	5	58	0			2	72	0		
44C2 × Kanred	3	52	0			2	62	0		
44D1 × Kanred	3	83	16	Shriveled.	19.2	2	82	3	Badly shriveled.	3.7
44G1 × Kanred	5	154	57	Plump.	37	2	98	4	Slightly shriveled.	4.1

## FIRST-GENERATION PLANTS, 1927

As a result of the second back cross, there were grown of the Hybrid 128 × Rosen combination 2 families consisting of 13 individuals; of Kanred × Dakold, 5 families, of 16 individuals; and of Kanred × Rosen, 4 families, of 22 individuals. As a result of selfing the plants of the first back-crossed generation,  $WR_1W_1$ , one family of each of the first two crosses and two of the third were grown, producing 6, 12, and 22 plants, respectively. Table 8 presents a summary of the data obtained on 11  $WR_1W_1W_1$  families, 4  $WR_1W_2$  families, and the two wheat parents.

TABLE 8.—Segregation for hairy neck, kernel color, fertility, kernel length, and kernel development in wheat × rye back-crossed families, 1927

Cross, generation, and parent	Plant ancestor	Family	Total plants	Plants with hairy neck of indicated degree				Plants with kernels of indicated color		Fertility		Kernel length		Plants with greater kernel length than wheat parent		Plants with indicated kernel development		
				Sterile plants	Heavy and medium	Light	None	Red	White	Average of kernels per spikelet	Plants with fewer kernels than wheat parent	Kernel length	Plants with greater kernel length than wheat parent	Plump and medium	Slightly shriveled	Badly shriveled		
Hybrid 128 × Rosen <sup>a</sup>	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
WR <sub>1</sub> W <sub>1</sub>	42A1					1		11		0.23		6.9						
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>		98B	11		3			4	7	.02-81	11	5.6-8.2	6	6	2	3		
WR <sub>1</sub> W <sub>1</sub>	42C1							1		.04		6.0						
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>		98Bd	12					1	1	.97-1.00	2	6.0-6.9	1					
WR <sub>1</sub> W <sub>2</sub>	42A1		6	1	2	1	4	4	1	.62-1.43	6	7.8-1.1	1	5				
Hybrid 128 × Kanred			4				4			41.42-1.86	5	8-6.5	3					
Kanred × Dakold:																		
WR <sub>1</sub> W <sub>1</sub>	43A1				1			1										
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>		99B	1		1			1		.04		17.0	1	1				
WR <sub>1</sub> W <sub>1</sub>	43B1					1		1										
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>		99Ba	3	2	1		2	1		.34	3	6.1	1				1	
WR <sub>1</sub> W <sub>1</sub>	42B2					1		1										
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>		99Cb	1	1			1			0								
WR <sub>1</sub> W <sub>1</sub>	43C1					1		1		.38		7.1		1				
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>		99Ac	9		1		8	9		.66-1.68	9	6.6-7.5	2	6	1	2		
WR <sub>1</sub> W <sub>1</sub>	43D3							1										
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>		99Cf	2		1		1	2		.05-97	2	7-7.0	1	1			1	
WR <sub>1</sub> W <sub>2</sub>	43C1		12	3		3	9	9		45-1.78	1	6.7-8.3	2	5	4			
Kanred × Rosen:																		
WR <sub>1</sub> W <sub>1</sub>	44B1						1	1		.11		5.7		1				
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>		400Aa	13				13	13		.66-1.87	8	6.3-7.5	6	11	1	1		
WR <sub>1</sub> W <sub>1</sub>	44B2						1	1										
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>		400Cb	1				1			0								
WR <sub>1</sub> W <sub>1</sub>	44D					1		1		.07		7.3					1	
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>		400Cc	3				3	3		.49-1.44	3	6.6-7.7	1	2			1	
WR <sub>1</sub> W <sub>1</sub>	44G					1		1		.08		6.8				1		
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>		400Dd	5	1			5	4		0-1.06	5	7.0-7.9	4	2			2	
WR <sub>1</sub> W <sub>1</sub>	44A2					1		1		.05		7.0						
WR <sub>1</sub> W <sub>2</sub>	44A2		10			2	8	8		.61-1.92	6		1	3	4	3		
WR <sub>1</sub> W <sub>1</sub>	44G1					1		1		.08		6.8						
WR <sub>1</sub> W <sub>2</sub>		44G1	12			2	10	12		.01-1.18	12	6.2-8.5	6	4	6	2		
Kanred			4				4	4		1.51-1.91		6.7-6.9		4				

<sup>a</sup> On one plant in this family no data on kernel color were recorded.<sup>b</sup> At top of peduncle.

In these second back-crossed families there was segregation for hairy neck, color of kernel, length of spike and kernel, and fertility. Similar segregation occurred in the WR<sub>1</sub>W<sub>2</sub> generation. Variation in length of spike in these generations is shown in Figures 4 and 5. Hairy-necked plants were always fewer in number than glabrous-necked plants, and red-kerneled plants were always fewer in number than white-kerneled plants. This relation was true likewise of long-spiked and long-kerneled plants, and their respective allelomorphs.

The fertility of all plants in nine families was below the minimum recorded for the respective parent plants. In one Kanred × Dakold WR<sub>1</sub>W<sub>2</sub> family of 12 plants (43C1), only one plant was below the parent minimum. Evidently this family had reverted almost completely to the wheat-parent type. Segregation for hairy neck and length of kernel was still occurring. Length of spike was within the

range of the Kanred parent, but height of plant was below that of the parent, probably as a result of bagging. Nearly all families

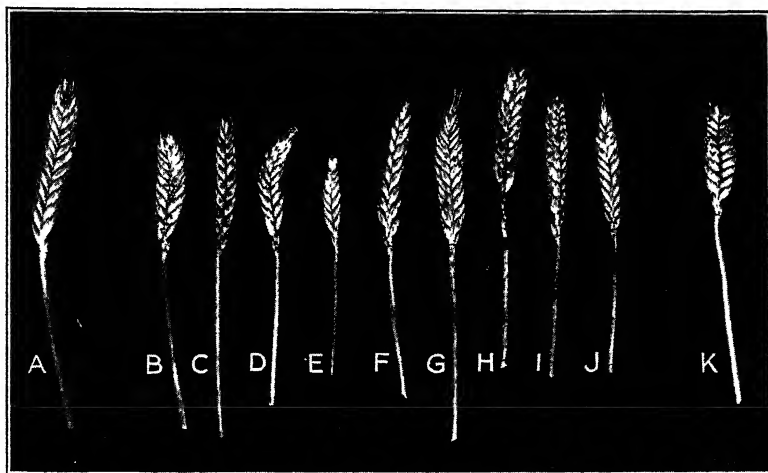


FIGURE 4.—Second back-cross first-generation hybrids and parents. A,  $WR_1W_1$  (same as fig. 3, A, b); B–J, head types resulting from the second back cross; K, Hybrid 128 wheat. Note variability resulting when plant with head type such as A is used for back crossing

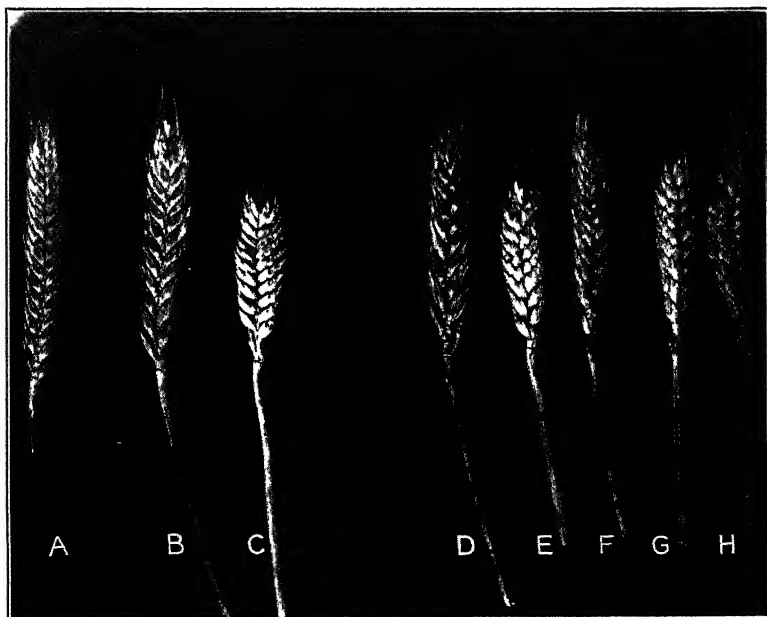


FIGURE 5.—Parents, first back-cross first-generation hybrid, and selfed first back-cross first-generation hybrids of Hybrid 128 wheat × Rosen rye back crosses: A,  $F_1$  hybrid; B,  $WR_1W_1$  (same as fig. 3, A, b); C, Hybrid 128 wheat; D–H,  $WR_1W_2$  population. Note type of variation when selfed

contained plants with length of kernel more than that of the parental maximum.



An interesting segregant (fig. 6, C) appeared in the  $WR_1W_1W_1$  generation of Hybrid 128  $\times$  Rosen family 98Bd. This family consisted of two plants, one of which seemed to be identical with plants of Hybrid 128 except that it had angular red kernels. The other plant was slightly larger with slightly longer white kernels. (Fig. 6, B.)

Most families had plants with more or less badly shriveled kernels. Endosperm development has been found to parallel closely the chromosome content of the fusion nucleus. The cytologic relationships are discussed in another paper by the author (5).

SECOND BACK-CROSS SECOND-GENERATION AND FIRST BACK-CROSS THIRD-GENERATION PLANTS. 1928

Seed was selected from plants having rye characters and from plants without apparent rye characters in order to get true-breeding

strains and to recover parental types. In each case plants of comparatively high fertility were used.

In 1928 6 families of the Hybrid 128  $\times$  Rosen combination, 3 of the Kanred  $\times$  Dakold, and 6 of the Kanred  $\times$  Rosen were grown in the  $WR_1W_1W_2$  generation; and 1, 2, and 3 families, respectively, of the same combinations were grown in the  $WR_1W_3$  generation. A total of 941 hybrid plants and 80 parent plants was studied.

The results from 10 hybrid families, and single families from each of the 2 wheat parents are presented in Table 9.

Two families each of the Kanred  $\times$  Dakold and Kanred  $\times$  Rosen crosses, both in the  $WR_1W_1W_2$  generation, had regained the fertility of the wheat parent. The range of length of spike of all was nearly identical with that of the parental range, and none had hairy neck. In all four families the maximum length of kernel was more than that of the parental maximum, however, showing that rye influence still remained. The percentage of long-kernelled plants (average length of kernel 6.9 mm. or more) in these families ranged from 17.9 to 56.5. A minority of plants with the rye character was found, except in one family. No true-breeding hairy-necked families occurred in either of these families nor in the Hybrid 128  $\times$  Rosen back cross. Nor were any true-breeding red-kernelled families found in the latter.

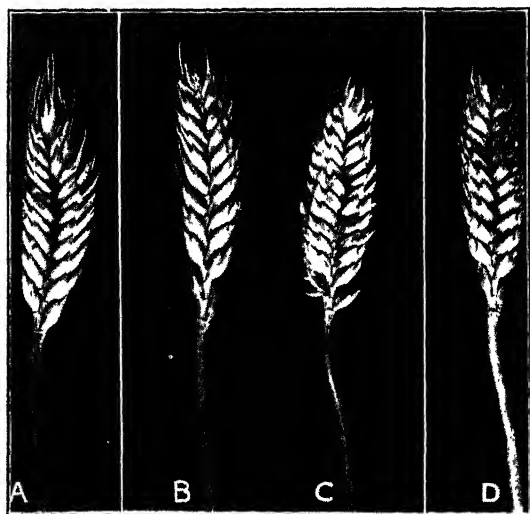


FIGURE 6.—Second back-cross second-generation hybrids and parents. A,  $WR_1W_1$  parent head (same as fig. 3, A, c); B and C, wheatlike heads of hybrid plants from the second back cross,  $WR_1W_1W_1$ ; D, Hybrid 128 wheat. Seed from the same plant as C, as the result of selfing, produced white-kernelled and red-kernelled plants; white-kernelled plants were identical with Hybrid 128. Selfed seed from the same plant as B, although white kernelled, produced hairy-necked and nonhairy-necked plants

TABLE 9.—*Segregation for hairy neck, kernel color, fertility, kernel length, and kernel development in wheat × rye back-crossed families, 1928*

Cross, generation, and parent	Plant ancestor, number	Family number	Total plants	Sterile plants	Plants with hairy neck of indicated degree			Plants with kernels of indi- cated color		Fertility		Kernel length	Plants with greater kernel length than wheat parent	Plants with indicated kernel de- velopment		
					Heavy and medium	Light	None	Red	White	Average of kernels per spikelet	Plants with fewer ker- nels than wheat parent			Plump and medium	Slightly shriveled	Badly shriveled
Hybrid 128×Rosen	No	No.	No.	No.	No.	No.	No.	No.	No.	No.	Mm	No.	No.	No.	No.	
WR <sub>1</sub> W <sub>1</sub>	42A1					1		1	0	23		6.9			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>	98B4					8			13	.63		8.2			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>2</sub>	98B4	20	2				10	4	1	0-2.18	12.5	0-7.4	11	8	2	8
WR <sub>1</sub> W <sub>1</sub>	42C1						1	1		.04		6.0			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>	98B3B						1	1		.97		6.0			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>2</sub>	98Bd3	114				114	32	82	12-1.88	63.5	1-6.4	15	68	26	20	
WR <sub>1</sub> W <sub>1</sub>	42C1						1	1		.04		6.0			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>	98Bd4						1	1		1.00		6.9			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>2</sub>	98Bd4	60	2	5	11	44	58	0-1.52	50.5	1-7.0	24	29	10	19		
WR <sub>1</sub> W <sub>1</sub>	42A1						1	1		.23		6.9			1	
WR <sub>1</sub> W <sub>2</sub>	42A1-1					1		1		1.27					1	
WR <sub>1</sub> W <sub>2</sub>	42A1-1	58	2	21	4	33	19	37	0-2.40	21	5.8-7.7	54	40	3	13	
Hybrid 128 × anred × Dakold:		30					30		30	142-2.77		5.2-5.9		30		
WR <sub>1</sub> W <sub>1</sub>	43C1					1		1		.38		7.1			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>	99Ac1						1	1		1.16		6.7			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>2</sub>	99Ac1	67				67	67	1.43-2.54	0.5	9-7.5	39	65	1	1		
WR <sub>1</sub> W <sub>1</sub>	43C1					1		1		.38		7.1			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>	99Ac7					1		1		1.33		7.5			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>2</sub>	99Ac7	48		5	7	36	48	14-1.86	20	5.7-7.9	21	35	11	2		
WR <sub>1</sub> W <sub>1</sub>	43C1					1		1		.38		7.1			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>	99Ac8						1	1		1.56		7.0			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>2</sub>	99Ac8	56				56	56	1.35-2.75	0.5	5-7.3	22	51	4	1		
Kanred × Rosen.																
WR <sub>1</sub> W <sub>1</sub>	44B1						1	1		.11		5.7			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>	400Aa1						1	1		1.66		6.3			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>2</sub>	400Aa1	64				64	64	1.34-2.47	0.5	8-7.1	12	55	8	1		
WR <sub>1</sub> W <sub>1</sub>	44B1						1	1		.11		5.7			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>	400Aa5						1	1		1.38		7.0			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>2</sub>	400Aa5	62				62	62	1.38-2.67	0.6	0-7.2	17	52	7	3		
WR <sub>1</sub> W <sub>1</sub>	44D					1		1		.07		7.3			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>1</sub>	400Cc1						1	1		1.38		7.7			1	
WR <sub>1</sub> W <sub>1</sub> W <sub>2</sub>	400Cc1	55	1			54	54	0-2.78	12	6.7-7.5	28	38	8	7		
Kanred		35				35	35	1.23-2.50		6.0-6.7			34		1	

<sup>a</sup> Dull white.<sup>b</sup> Including two grassy dwarfs.<sup>c</sup> Pale red.<sup>d</sup> Including one grassy dwarf.

The progeny of the red-kerneled plant of the Hybrid 128 × Rosen WR<sub>1</sub>W<sub>1</sub>W<sub>1</sub> family, 98Bd3, did not breed true for kernel color, but from all indications this plant was homozygous except for this character. (Fig. 7.) Out of 114 plants, 32 were redkerneled and 82 whitekerneled, a ratio of 1 red to 2.56 white. Seventy-eight per cent of the red-kerneled plants and 46.3 per cent of the white-kerneled plants were below the parental minimum of fertility. There was a definite tendency for red-kerneled plants to have longer kernels than the white-kerneled plants. The family of the white-kerneled sib of the red-kerneled plant 98Bd4 (fig. 6, B) bred true for white kernel but segregated for all other characters studied.

Segregating families produced a larger number of plants with shriveled kernels than did parental-type families.

RYE CHARACTERS AND FERTILITY IN SECOND BACK-CROSS THIRD-GENERATION  
AND FIRST BACK-CROSS FOURTH-GENERATION PLANTS. 1929

An effort was made to get true-breeding wheat types having one or more of the rye characters—hairy neck, red kernel color, long kernel, and winter hardiness. Fifteen Hybrid 128×Rosen, five Kanred×Dakold, and four Kanred×Rosen families were grown in the  $WR_1W_1W_3$  generation. Seven Hybrid 128×Rosen and two Kanred×Dakold families were grown in the  $WR_1W_4$  generation. There were 2,193 hybrid and 119 parent plants harvested and studied in 1929.

Data on 19 families, together with similar data for ancestral plants and parent varieties, are presented in Table 10. One family, 98B4-2, bred true for hairy neck, and one, 42A1-1-37, bred nearly true for red kernel. Both were Hybrid 128×Rosen back crosses, the first a  $WR_1W_1W_3$  and the second a  $WR_1W_4$ .

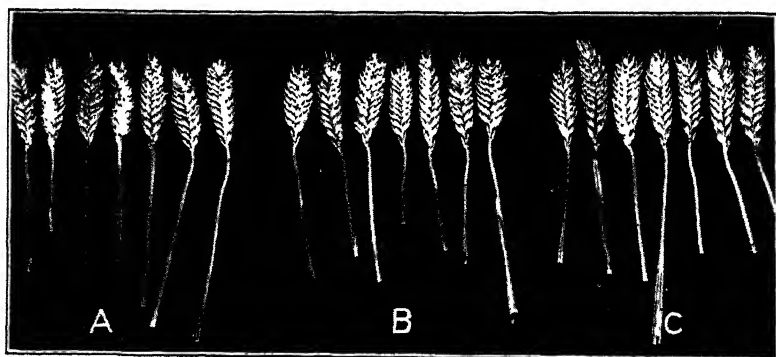


FIGURE 7.—Second back-cross second-generation heads resulting from selfing plant represented by Figure 6, C, and heads of Hybrid 128 wheat for comparison. A, Hybrid 128 wheat; B, white-kerneled segregates; C, red-kerneled segregates. Note close similarity of segregates B and C to Hybrid 128

The hairy-necked family originated from a plant with unusually heavy pubescence extending down the peduncle about 2 inches below the spike. The other three characters, fertility, color, and length of kernel, were segregating in this family. Twenty plants were below the parental minimum of fertility, and one plant, a semidwarf, was sterile.

Family 42A1-1-37, nearly true breeding for red kernels, originated from a red-kerneled line of ancestors. Other characters besides kernel color were segregating in this family. Twenty-one of 53 plants were sterile, 1 being a semidwarf. One of the 32 fertile plants was white kerneled. It seems likely that this plant was not a mechanical mixture, but resulted from the elimination of the rye factor or factors responsible for red color.

Ten red-kerneled plants from the  $WR_1W_1W_2$  Hybrid 128×Rosen family, 98Bd3, which was segregating for kernel color but otherwise true breeding, were grown. Not one true-breeding red-kerneled family resulted. In nine families there was a preponderance of white-kerneled plants as usual, but in one family, Bd3-19, there were 22 red to 14 white. The most fertile red-kerneled plants were chosen as parents for these 10 families. It is possible that true-breeding plants were unconsciously eliminated. The kernels of the red-kerneled plants were fairly well developed in most cases, although more or less angular; the kernels of the white-kerneled plants were mostly plump and well filled.

TABLE 10.—*Segregation for hairy neck, kernel color, fertility, kernel length, and kernel development in wheat × rye back-crossed families, 1929*

Cross, generation, and parent	Plant ancestor number											Plants with hairy neck of indicated degree		Plants with kernels of indicated color <sup>a</sup>		Fertility		Plants with greater kernel length than wheat parent			Plants with indicated kernel development		
		Family number	Total plants		Sterile plants	Dwarf plants	Heavy and medium			Light	None	Red	White	Average of kernels per spikelet	Plants with fewer kernels than wheat parent	Kernel length	Plants with greater kernel length than wheat parent	Plump and medium					
			Slightly shriveled	Badly shriveled																			
Hybrid 128 × Rosen:	No	No	No	No	No	No	No	No	No	No	No	No	Mm.	No	Mm.	No	No	No	No				
W <sub>R</sub> W <sub>1</sub>	42A1							1			1		0.23		6.9								
W <sub>R</sub> W <sub>1</sub> W <sub>1</sub>	98B4							1				b1			8.2		1						
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	98B4-2							1					25		7.4								
W <sub>R</sub> W <sub>1</sub> W <sub>3</sub>	(4)	30	1	1		24	6				4	25	0-2.76		20.5-7-7.7	26	28		1				
W <sub>R</sub> W <sub>1</sub>	42C1										1		0.4		6.0								
W <sub>R</sub> W <sub>1</sub> W <sub>1</sub>	98Bd3										1		97		6.0		1						
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	98Bd3-4	(4)	72								1		32		6.2		1						
W <sub>R</sub> W <sub>1</sub> W <sub>3</sub>	98Bd3-16	(4)	67								1		145		6.0		1						
W <sub>R</sub> W <sub>1</sub> W <sub>4</sub>	98Bd3-19	(4)	36								1		28	22-2.40	8.5-2-0.1		35	1					
W <sub>R</sub> W <sub>1</sub> W <sub>1</sub>	98Bd3-25	(4)	66								1		35		6.1		1						
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	98Bd3-20	(4)	61								1		18	72-2.77	11.3-5-6.5	1	32						
W <sub>R</sub> W <sub>1</sub> W <sub>3</sub>	98Bd3-36	(4)	75								1		14	63-2.63	2.5-5-6.2		20	1					
W <sub>R</sub> W <sub>1</sub> W <sub>4</sub>	98Bd3-44	(4)	70								1		14	58-2.5	5.4-1-6-0		17		2				
W <sub>R</sub> W <sub>1</sub> W <sub>1</sub>	98Bd3-59	(4)	68								1		139		5.8		1						
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	98Bd3-76	(4)	80								1		141	36-2.44	4.4-4-6-3	2	19		1				
W <sub>R</sub> W <sub>1</sub> W <sub>3</sub>	98Bd3-103	(4)	77								1		1.50		5.4		20						
W <sub>R</sub> W <sub>1</sub> W <sub>4</sub>	98Bd3	(4)	264								1		161	57-2.80	3.5-3-6.3	1	19		1				
Hybrid 128		80									1		1.50		5.4		20						
W <sub>R</sub> W <sub>1</sub>	42A1										1		23		6.9								
W <sub>R</sub> W <sub>1</sub> W <sub>1</sub>	42A1-1										1		1.27		7.0		1						
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	42A1-1-1	(4)	55	2		14	9	32	19	19	0-2	53	22	5.0-7.4	31	16	18	4					
W <sub>R</sub> W <sub>1</sub> W <sub>3</sub>	42A1-1-23	(4)	39			4	5	30					37	37-2.67	13.5-5-7.1	15	25	4	8				
W <sub>R</sub> W <sub>1</sub> W <sub>4</sub>	42A1-1-37	(4)	52	21	1	2	20	29	30	1	0-2	0.7	49	4.7-7.4	33	9	10	12					
Kanred × Dakold:																							
W <sub>R</sub> W <sub>1</sub>	43C1										1		38		7.1		1						
W <sub>R</sub> W <sub>1</sub> W <sub>1</sub>	99Ac7										1		133		7.5		1						
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	99Ac7-18	(4)	66								1		80		7.0		1						
W <sub>R</sub> W <sub>1</sub> W <sub>3</sub>	99Ac8										1		1.00-3.43		20.1-7.5	3	35		1				
W <sub>R</sub> W <sub>1</sub> W <sub>4</sub>	99Ac8-23	(4)	88		4						1		1.56		7.0		1						
W <sub>R</sub> W <sub>1</sub> W <sub>1</sub>	Kanred										1		1.88		7.3		1						
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>											1		1.23-3.25		15.5-7-7.2	1	41	2					
W <sub>R</sub> W <sub>1</sub> W <sub>3</sub>											1		1.48		7.4		41		3				
Kanred × Rosen:																							
W <sub>R</sub> W <sub>1</sub>	44B1										1		11		5.7				1				
W <sub>R</sub> W <sub>1</sub> W <sub>1</sub>	400Aa13										1		92		7.0		1						
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	400Aa13-36	(4)	37								1		1.72		7.2		1						
W <sub>R</sub> W <sub>1</sub> W <sub>3</sub>	44D										1		0.7		7.3		1						
W <sub>R</sub> W <sub>1</sub> W <sub>4</sub>	400Ccl										1		1.38		7.7		1						
W <sub>R</sub> W <sub>1</sub> W <sub>1</sub>	400Ccl-3	(4)	71								1		1.94		7.3		1						
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	400Ccl-7	(4)	56								1		1.67		7.2		1						
W <sub>R</sub> W <sub>1</sub> W <sub>3</sub>											1		1.41-2.43		5.6-8-7.8	18	33						

<sup>a</sup> Kernel color, fertility, kernel length, and kernel development were determined on approximately 20 plants in each family in (W<sub>R</sub>W<sub>1</sub>W<sub>4</sub>) F<sub>1</sub>.<sup>b</sup> Dirty white.<sup>c</sup> Very heavy.<sup>d</sup> Cross number same as F<sub>2</sub> family.<sup>e</sup> Total plants of all F<sub>2</sub> families of 98Bd3<sup>f</sup> Dull red.<sup>g</sup> Cross number same as F<sub>3</sub> family.

In 1929, parent varieties and parental strains generally produced plump, well-filled grain, but segregating families produced about the usual proportion of shriveled kernels.

RELATIONSHIP OF FERTILITY AND KERNEL LENGTH IN SECOND BACK-CROSS SECOND AND THIRD GENERATION FAMILIES AND FIRST BACK-CROSS THIRD-GENERATION FAMILIES, 1928 AND 1929

#### WHEATLIKE FAMILIES WITH RYE CHARACTERS

Although no wheatlike strain having true-breeding rye characters has been obtained so far as can be determined, the isolation of strains apparently with a single rye character, as well as others with two or more rye characters, affords opportunity to study the effect of the rye complement on fertility and length of kernel in the wheat plant. Table 11 presents data showing some of these relationships.

In 1927, the red-kerneled plant 98Bd3, of the Hybrid 128 × Rosen second back cross, to all appearances was identical with Hybrid 128, except for kernel color and slight differences in kernel shape. The selfed seed of this plant, in 1928, produced 32 red-kerneled and 82 white-kerneled plants, or a ratio of 1 red to 2.56 white. White kernel color in wheat is recessive, so that the segregation is not the same as that usually obtained in crosses between wheat varieties. The white-kerneled plants were identical in morphological characters with those of the parent, Hybrid 128, except for such variations as are normally found within the variety itself. The kernels of the red-kerneled plants were slightly longer on the average and more or less angular, instead of plump as in the white-kerneled plants and in Hybrid 128. Red-kerneled plants were also less fertile.

The mean fertility of the red-kerneled plants, expressed as the average number of kernels per spikelet, was  $1.03 \pm 0.047$ ; of the white-kerneled plants,  $1.37 \pm 0.022$ ; and of the parent plants,  $1.77 \pm 0.040$ . All hybrid and parent plants were bagged. The difference in fertility between the red-kerneled and white-kerneled plants was 0.30 in favor of the white-kerneled plants. This difference is 5.8 times its probable error. The factor, or determining element, for the red color apparently caused a reduction in fertility. A significant difference in fertility also was noted between the white-kerneled and parent plants, but this may have been due partly to differences in time of bagging or perhaps to soil variation.

In 1929, when all plants were grown without being bagged, the mean fertility of the white-kerneled plants was  $2.05 \pm 0.010$  and of the parent plants  $2.14 \pm 0.025$  kernels per spikelet. The difference of the means of the two classes of plants was 3.3 times the probable error. The mean fertility of the red-kerneled plants was but  $1.90 \pm 0.030$ , which is significantly less than that of the white-kerneled plants.

The increased length of kernel of the red-kerneled plants was not statistically significant in 1928, but there was a significant increase, amounting to four times the probable error, in 1929, when a larger number of plants was examined. There was no significant difference in kernel length between the white-kerneled plants and those of the parent variety in 1928, but in 1929 there was a difference of four times the probable error. In both years the fertility of the parent was significantly higher than that of the white-kerneled segregates.

The progeny of the white-kerneled sib plant 98Bd4 segregated for hairy neck and kernel length in 1928. The decrease in fertility and the increase in length of kernel of the hairy-necked plants, as compared with those of the nonhairy-necked plants, were positive and significant. The nonhairy-necked plants here were significantly lower in fertility than the wheat-parent plants. (Compare with Hybrid 128 parent.)

In family 98B4-2 all plants were hairy necked, but there was segregation for development of hairy neck, for kernel length, and for kernel color. A significantly greater length of kernel and a higher degree of sterility were found in plants classed as heavy and medium-heavy hairy neck than were found in plants having light hairy neck.

The light hairy-necked condition showed slight but not significant decrease in fertility when compared with the nonhairy-necked plants in redkerneled family 98B4-2. There was no significant difference in average length of kernel between the two groups.

The white-kerneled  $WR_1W_3$  family 42A1-23, segregating for hairy neck and kernel length, shows relationships similar to those of 98Bd4 for these two characters. Hairy necked and nonhairy necked plants occurred in a proportion similar to that found in the red-kerneled family 98B4-2.

The segregation in Hybrid 128 × Rosen second back-cross family 98Bd4, true breeding for white kernel, was 16 hairy neck to 44 glabrous (Table 9); the segregation in first back-cross second-generation family 42A1, segregating for color of kernel and hairy neck, was 3 hairy to 4 glabrous. (Table 8.) A hairy-necked selection, 42A1-1, produced 25 hairy neck to 33 glabrous. (Table 9.) The segregation in Kanred × Dakold back-cross family 99Ac7 was 12 hairy neck to 36 glabrous.

In 1928, Kanred × Dakold  $WR_1W_1W_2$  family 99Ac7, segregated for hairy-neck but otherwise was true breeding and wheatlike. The fertility of the hairy-necked plants averaged  $1.28 \pm 0.091$  kernels per spikelet compared with  $1.47 \pm 0.043$  kernels per spikelet for the nonhairy-necked plants. (Table 11.) The difference probably is significant, but does not appear to be so on account of the large probable error of the hairy-necked group, due to the high degree of sterility in many plants. Kernel length in the hairy-necked plants is significantly greater than in the nonhairy-necked group.

In 1929, a light hairy-necked selection of the same back cross, 99Ac7-18, produced but one hairy-necked plant in a family of 36. Mean fertility, length of kernel, and other characters of the nonhairy-necked plants were almost identical with those of the Kanred-parent family.

TABLE 11.—Mean fertility and kernel length in segregating and true-breeding families of wheat  $\times$  rye back crosses, 1928 and 1929

Cross, generation, segregator, and parent	Family	Segregating characters	Year grown	Total plants	Plants with hairy neck of indicated degree—			Plants with kernels of indicated color		Mean fertility (average of kernels per spikelet)			Mean kernel length	
					Heavy and medium	Light	None	Red	White	Mean	Probable error	Diff. $m^2$ P. E. $m$	Mean	Probable error
					Number	Number	Number	Number	Number	Number			Mm	Diff. $m^2$ P. E. $m$
Hybrid 128 $\times$ Rosen: WR <sub>1</sub> W <sub>1</sub> W <sub>2</sub> Red kernelled White kernelled	No. 98B43	Kernel color	1928	114			114	32	82	1.07			5.7	
				32						1.37			5.6	
				82						1.77			5.5	
Hybrid 128 $\times$ b WR <sub>1</sub> W <sub>1</sub> W <sub>2</sub> Red kernelled White kernelled	No. 98B43	Kernel color	1929	10						2.26			5.7	
				264			264	90	174	1.90			5.9	
				174						2.05			5.8	
Hybrid 128 $\times$ c WR <sub>1</sub> W <sub>1</sub> W <sub>2</sub> Hairy neck Nonhairy neck	No. 98B44	Hairy neck and kernel length	1928	41			41		41	2.14			5.9	
				60			42		58					
				16						.57			6.2	
Nonhairy neck WR <sub>1</sub> W <sub>1</sub> W <sub>2</sub> Light hairy neck Heavy and medium-heavy hairy neck	No. 98B4-2	Kernel color and kernel length	1929	43						1.05			5.8	
				30				4	25					
				6				1	5	2.05			6.8	
WR <sub>1</sub> W <sub>2</sub> Light hairy neck Nonhairy neck	No. 42A1-37	Hairy neck and kernel length	1929	52						1.27			6.9	
				11				39	1					
				9						.56			6.6	
WR <sub>1</sub> W <sub>2</sub> Hairy neck Nonhairy neck	No. 42A1-23	Hairy neck and kernel length	1929	19						.77			6.5	
				37				37						
				10						1.70			6.6	
WR <sub>1</sub> W <sub>2</sub> Hairy neck Nonhairy neck	No. 99A67	Hairy neck	1928	48						2.04			6.1	
				12				48						
				36						1.28			7.1	

W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	1929	36	1	35	36	2.00	±.030	6.7 ±.037	0.1
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	1928	67		67	67	2.06	±.020	6.8 ±.021	
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	1928	56		56	56	2.01	±.025	6.7 ±.038	
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	1929	43		43	43	1.77	±.035	6.8 ±.030	
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	1929	35		35	35	1.80	±.014	6.8 ±.019	
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	1928	64		64	64	1.98	±.010	6.5 ±.024	
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	1928	62		62	62	1.82	±.033	6.5 ±.027	
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	1928	54		54	54	1.64	±.043	6.8 ±.032	
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	1929	34		34	34	1.83	±.033	7.0 ±.036	
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	1929	33		33	33	1.88	±.027	7.2 ±.028	
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	1928	10		10	10	1.72	±.034	6.3 ±.018	
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	1928	25		25	25	1.63	±.050	6.6 ±.012	
W <sub>R</sub> W <sub>1</sub> W <sub>2</sub>	1929	44		44	44	2.02	±.020	6.8 ±.020	

<sup>a</sup> Two grassy dwarfs additional.  
<sup>b</sup> One grassy dwarf additional.  
<sup>c</sup> Plus two dwarfs.

<sup>d</sup> Progeny of 10 red-kerneled plants from F<sub>2</sub> family 981d3.

<sup>e</sup> Dull red.

<sup>f</sup> Including one semidwarf.

<sup>a</sup> Probable error of difference of probable errors  $\sqrt{(P.E.)^2 + (P.E.)^2}$ .

<sup>b</sup> Plants bagged to prevent contamination by foreign pollen.

<sup>c</sup> Plants not bagged.



## WHEATLIKE FAMILIES OF PARENT TYPE

In 1928, Kanred  $\times$  Dakold  $WR_1W_1W_2$  family 99Ac8, having 56 plants, apparently was again completely fertile, since it was higher in fertility than the Kanred parent. However, length of spike and length of kernel were slightly greater than in the Kanred parent. (Table 11.)

In 1929, two selections from the same family, 99Ac8-23 and 99Ac8-35, produced families both of which were slightly lower in fertility than the Kanred parent. Family 99Ac8-23 had four grassy dwarfs, which normally are not found in Kanred. Dwarfs may indicate some sort of chromosome irregularity.

In 1928, Kanred  $\times$  Rosen  $WR_1W_1W_2$  families 400Aa1 and 400Aa6 were true breeding and of Kanred type. Height of plant, length of spike and kernel, fertility, and other plant characters were normal. (Table 11.) These selections evidently had returned to the parent type.

## DISCUSSION

The varieties of common wheat used in this experiment cross quite readily with Rosen and Dakold ryes. The  $F_1$  was completely sterile when the plants were protected from contamination by foreign pollen. Unprotected plants yielded up to about 0.6 per cent of viable kernels. These results agree with those of Jesenko (8), Meister (12), Leighty and Taylor (11) and others. Such results are not unexpected in view of the extent of chromosome incompatibility found in wheat ( $n^7=21$ )  $\times$  rye ( $n=7$ ) hybrids. The small set of fertile seed obtained from back crossing or from open pollination shows, however, that a small proportion of viable ovules are formed. It seems likely that both male and female gametes of similar constitution may be produced. The chances of two gametes capable of fertilization coming together would be so remote that self-sterility is complete for all practical purposes. Watkins (17) concluded from a study of hybrids of a cross of *vulgare*  $\times$  *turgidum* wheats that sterility is due in most cases to lack of functional pollen. The situation in wheat  $\times$  rye hybrids is not strictly comparable but is probably similar; therefore, with relatively high mortality in the pollen cells, the chance of obtaining selfed seed in  $F_1$  wheat  $\times$  rye plants is extremely small. No such seed has been obtained. When normal pollen is used a small degree of fertility is displayed.

When the back crosses of the several wheat  $\times$  rye hybrids were grown the plants from each parent pair were more wheatlike than those from the  $F_1$  parent, but there was variation among them. Certain plants exhibited more rye characters than others, while an occasional plant was very similar to the wheat parent. Such results might be expected from random distribution of the chromosomes. Kihara (9) and Thompson (16) report that random distribution of the chromosomes occurs in  $F_1$  wheat  $\times$  rye hybrids. However, there is in operation another type of chromosome distribution, which results from the formation of somatic gametes. This is discussed more fully by the author in a paper on a cytological study of this same wheat  $\times$  rye  $\times$  wheat hybrid material (5). The different types of plants obtained showed distinct variability in the chromosome constitution of the ovules of the  $F_1$  wheat  $\times$  rye.

<sup>7</sup> n=any number of chromosomes.

The degree of sterility was reduced by back crossing, but variability of plant characters was increased. Nineteen viable  $WR_1W_1$  kernels produced 14 completely sterile plants; 51 viable  $WR_1W_1W_1$  kernels produced only 8 completely sterile plants. The repeatedly back-crossed plants became more wheatlike with increased fertility, presumably a direct result of the elimination of rye chromosomes. A similar return to more wheatlike forms was noted in back-crossed material that was selfed.

Several lines were obtained from the second back cross that apparently closely conformed to the wheat-parent type. They were wheatlike and fully fertile.

The white-kerneled plants segregating from the red-kerneled wheatlike strain 98Bd3, of the Hybrid 128  $\times$  Rosen back cross, appeared to be identical with Hybrid 128 both in plant characters and fertility. The red-kerneled plants were somewhat reduced in fertility. The kernels of the latter were angular and on the average slightly longer than those of Hybrid 128. Length of spike also was slightly increased. The factor for hairy neck similarly reduced fertility and slightly modified the expression of plant characters. Apparently the presence of rye factors or of elements carrying such factors sets up a state of unbalance which interferes with the normal functioning of the wheat-chromosome system.

It has been mentioned that the red-kerneled  $WR_1W_1W_1$  Hybrid 128-like plant, 98Bd3, was the ancestor of a strain wheatlike in all characters but kernel color. The  $WR_1W_1W_2$  family grown in 1928 produced a ratio of one red kernel to 2.56 white kernels. Red-kerneled plants from this progeny produced similar results in 1929. The white-kerneled or recessive plants were always in preponderance. In explaining this apparent reversal of dominance, chromosome constitution must be considered. In one  $WR_1W_1W_3$  family the white-kerneled plants were found to have 42 chromosomes, and the red-kerneled 43 and 44 (5). The type of segregation obtained evidently resulted from the various recombinations of 21 and 22 chromosome gametes, the red color being carried by the extra chromosome. Similar types of segregation occurred in most hairy-necked and long-kerneled progenies. It is likely that the rye factors producing these characters were added as whole chromosomes.

The occurrence of a nearly true-breeding red-kerneled line and of an apparently homozygous hairy-necked line (in  $WR_1W_1W_3$ ) gives some indication of the possibility of producing wheat-rye strains constant for specific rye characters. It is possible that rye elements may be brought into the wheat-chromosome system by (1) the introduction of rye genes by crossing over, (2) the substitution of a rye pair of chromosomes for a wheat pair, or (3) the addition of rye chromosomes to the normal wheat complement. The limited and loose pairing noted in wheat  $\times$  rye hybrids does not appear favorable to the transfer of rye genes to wheat by crossing over even if it should be definitely established that the pairing involved a wheat and a rye chromosome. Chromosome substitution has not been demonstrated. The addition of a single chromosome pair may be possible, since pairing and fairly normal reproductive cell divisions were noted in 44-chromosome plants of the red-kerneled progeny of 98Bd3-16.

## SUMMARY

Crosses were made between three 42-chromosome and four 28-chromosome wheats and Rosen and Dakold rye. The 42-chromosome varieties were fertilized quite easily with rye pollen, while the 28-chromosome varieties were fertilized with difficulty or not at all.

The  $F_1$  wheat $\times$ rye hybrids were completely self-sterile. Both dominant and intermediate wheat or rye characters were present, but in general the  $F_1$  plants were more wheatlike than rye-like.

The  $F_1$  plants produced a small percentage of viable kernels upon back crossing. The  $F_1$  hybrids of Hybrid 128 and Kanred wheats crossed with Dakold and Rosen ryes were back crossed to their respective wheat parents. These back crosses were again back crossed to the same wheat parents. Genetic and cytologic studies were made of the back crosses of Hybrid 128 $\times$ Rosen; genetic studies only were made of the back crosses of Kanred $\times$ Dakold and Kanred $\times$ Rosen.

Selfed as well as back-crossed plants in the first back-cross first generation ( $WR_1W_1$ ) and in the second back-cross first generation ( $WR_1W_1W_1$ ) and second generation ( $WR_1W_1W_2$ ) were covered with manila-paper bags to prevent contamination with foreign pollen. Bagging tends to reduce the normal fertility. In a comparison between bagged and unbagged plants, in 1928, bagging reduced fertility 17.9 per cent in Hybrid 128 and 19.7 per cent in Kanred.

Fertility was increased by back crossing with the parent wheat, and progeny plants became more wheatlike. Viable seeds from the back cross on  $F_1$  produced approximately 74 per cent of sterile plants. Viable seeds from the back crosses on first-generation back-crossed plants ( $WR_1W_1$ ) produced about 16 per cent of sterile plants. The progeny of selfed plants also tended to become more wheatlike. Parent types were recovered in the second back-cross second and third generations.

Plants that had red kernel color, hairy neck, and long kernel from wheatlike families of Hybrid 128 $\times$ Rosen back crosses usually exhibited reduced fertility. Those with red kernels appeared least fertile. Plants having light hairy neck were more fertile than those having heavy hairy neck. A wheatlike, nearly homozygous, red-kernelled progeny and an apparently homozygous hairy-necked progeny were obtained, indicating the possibility of producing constant wheat $\times$ rye strains.

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# A CYTOLOGIC STUDY OF WHEAT×RYE HYBRIDS AND BACK CROSSES<sup>1</sup>

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## INTRODUCTION

In a genetic study of wheat×rye hybrids and back crosses reported by the writer (4),<sup>3</sup> varieties of the 21-chromosome wheats were crossed with varieties of rye ( $n=7$ ).<sup>4</sup> Complete sterility was noted in  $F_1$  hybrids. A low percentage of fertility was obtained on back crossing with the wheat parent. The plants thus obtained were more wheatlike than those of the  $F_1$  hybrid, but they showed variation. The  $WR_1W_1$ <sup>5</sup> plants obtained in the first back cross were again back crossed with the same parent. From the second back cross,  $WR_1W_1W_2$  generation, a wheatlike line was obtained which segregated in a ratio of 1 red-kerneled to 2.56 white-kerneled plants. In similar strains from both  $WR_1W_3$  and  $WR_1W_1W_3$  generations, kernel color and "hairy neck" appeared more or less constant. It is evident that a knowledge of the chromosome relationships of the hybrid progeny is necessary to explain the phenomena observed.

## REVIEW OF LITERATURE

The cytology of wheat×rye hybrids was first studied in 1920 by Kihara (7). He found the somatic chromosome number to be 28. From 1 to 3 bivalents in loose combination and from 28 to 22 univalents were noted in the metaphase. About 26 per cent of the cells counted contained only univalents. Chance distribution of univalents followed by abnormalities in later stages of division was the rule.

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<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 362.

<sup>4</sup>  $n$ =any number of chromosomes.

<sup>5</sup> The designation  $WR_1W_1$  used throughout this paper denotes that the  $F_1$  of the wheat×rye cross (written as  $WR_1$ ) has been back crossed with the wheat parent,  $W$ , resulting in the plant or series of plants in question. The formula without the subscript or generation numeral, as  $WR_1W$ , refers to the crossed kernels, or  $P_1$ , of the back cross in question. The generation of the back cross is designated by the usual subscript numeral, as  $WR_1W_1$ ,  $WR_1W_2$ , etc.  $WR_1W_2$  indicates that the plant  $WR_1W_1$  has been selfed producing a second generation. Likewise,  $WR_1W_1W_1$  indicates that the original  $F_1$  hybrid has been back crossed with wheat and the resulting hybrid again back crossed with the wheat parent, producing the plant thus designated. When this plant is selfed the resulting plant is indicated by  $WR_1W_1W_2$ , and later selfed generations by the usual subscript numerals 3, 4, etc. In crosses involving unequal chromosomes, or chromosomes that do not pair, plants from the back cross on  $F_1$  usually vary somewhat in type, unlike the  $F_1$  plants of ordinary crosses, owing to variability in constitution of the gametes of the hybrid.

Zalensky and Doroschenko (18) found that the chromatin thread uncoiling after synapsis seldom appears double, which they believed indicated lack of conjugation. General phenomena were similar to those observed by Kihara.

Melburn (10), in a comparative study of the thin thread or spireme stage in the early meiotic prophases in the parents and in the  $F_1$  wheat $\times$ rye hybrid, found identical phenomena up to the first contraction or synzinesis stage; i. e., the spireme, which is believed to be composed of univalent chromosomes joined end to end, appears as a double thread in both. This appearance can have no significance in relation to chromosome pairing because it is largely absent in the hybrid. Pairing in the parents begins by the formation of loops (made up of pairs of chromosomes still comparatively long and thin), whose sides approach and anastomose and twist. There is some loop formation in the hybrid, but the loops do not anastomose or twist.

Thompson (15) found the same number of bivalent and univalent chromosomes as did Kihara, with similar phenomena in later stages. He believed that the pairing he observed was due to autosyndesis or self-pairing of wheat chromosomes.

Longley and Sando (9) observed no pairing in the cross *Triticum vulgare* $\times$ *Secale cereale*, but in the cross *T. vulgare* $\times$ *S. montanum* (a species closely related to *S. cereale*) from one to three bivalents were seen. However, even in the latter hybrid, bivalents were observed only rarely, and lack of pairing was the rule.

Plotnikov (11) found somatic chromosome numbers of 42 to 49 in  $F_3$  plants from spontaneous wheat $\times$ rye hybrids. Sterile  $F_1$  plants were pollinated with wheat. Although the  $F_1$  and  $F_2$  were not examined, the author concluded that  $F_1$  gametes may contain from 21 to 28 chromosomes.

#### MATERIAL

Most of the material studied comprised the hybrid and back-crossed hybrid progeny of Hybrid 128 wheat (*Triticum compactum* Host.) $\times$ Rosen rye (*Secale cereale* L.). The material was grown at Davis, Calif., in 1927 and 1928, and at Berkeley, Calif., in 1929. Back crosses involving Kanred wheat (*T. vulgare* Vill.), Red Winter spelt (*T. spelta* L.), and Dakold rye (*S. cereale*) were also investigated to some extent. Back crosses were made only with the wheat parents.

Hybrid 128 is a fully constant, winter-habit, white-kerneled, short, strong-strawed wheat developed at the Washington Agricultural Experiment Station (2).

Rosen rye is a winter variety with strong, faintly purple straw, and a long, broad, fairly dense spike. In the experiments here reported kernel color usually was pale red or yellowish, but gray and green kernels nearly always were present. The peduncle just below the spike is more or less heavily pubescent for a distance of about 2 inches. The term "hairy neck," first used by Leighty and Taylor (8), will be used to designate this character.

Somatic and pollen mother-cell chromosomes of the parents and  $F_1$  of Hybrid 128 $\times$ Rosen were studied. Somatic chromosome numbers were determined in  $WR_1W_1$  back-crossed plants of four crosses listed in Table 2. Somatic and pollen mother-cell chromosomes were examined extensively in Hybrid 128 $\times$ Rosen,  $WR_1W_3$ , and  $WR_1W_1W_3$ .

It would have been desirable to make a similar study of the plant ancestors of the hybrid lines studied intensively in  $F_3$ , but this was not done owing to lack of suitable preserved material.

## METHODS

Root tips for chromosome counts were collected from plants of the  $F_1$ ,  $WR_1W_1$ ,  $WR_1W_1W_1$ , and certain families of the  $WR_1W_3$  and  $WR_1W_1W_3$  generations. Plants from which root tips were to be collected were grown in pots to permit a root development sufficient to supply a suitable number of root tips. This required from two to three months. The earlier samples were collected from plants about 6 weeks old. After collecting the root tips, the plants were transplanted in the field.

The root tips collected in 1926 and 1927 were fixed in chrom-acetic-urea, washed, run through the various solutions of alcohol and xylol, and embedded in paraffin in the usual way. In the fall and winter of 1928-29 nearly the same schedule was used, except that S. Nawashin's fixative, as modified by Karpetschenko, was used.<sup>6</sup> Better fixation with greater definition of chromosomes was obtained with chrom-acetic-formalin than with chrom-acetic-urea.

In the plants having 42 or more chromosomes, greatest success was obtained from sections cut to a thickness of  $14\mu$ . In  $12\mu$  sections a disproportionately large number of metaphase cells were cut. Heidenhain's iron haematoxylin was used to stain the sections.

Anthers were gathered from fresh material brought to the laboratory. Aceto-carmin was used to stain the temporary slides for pollen mother cells. Slides containing suitable stages were sealed in by a preparation of equal parts of paraffin and gum mastic. Slides properly prepared were preserved in good condition from three to six months.

Most of the material for the temporary slides was collected and examined between 4.30 and 9 a. m. Moderately high to high temperatures were found unfavorable for good division figures. Such temperatures frequently occur at the time of meiosis in cereals where this work was done. The pollen mother cells then pass their stages rapidly. With high temperatures and a serious depletion of soil moisture, the pollen mother cells disintegrate.

## CYTOLOGIC INVESTIGATIONS

In normally fertile and constant strains or races of plants the chromosomes pair in meiosis. The importance of a study of cytologic relations is evident in crosses where sterility is complete in the initial cross and where back crossing ordinarily is the only method of regaining fertile strains in which may be combined the desired species characters. Chromosome pairing, chromosome numbers, distribution in meiosis, and tetrad and pollen formation were studied in parents, hybrids, and back crosses.

Most of the chromosome studies were confined to the  $F_1$ , the  $WR_1W_1$  back cross, and the  $WR_1W_3$  and  $WR_1W_1W_3$  back crosses of Hybrid 128  $\times$  Rosen.

<sup>6</sup> Fixative. Solution A: 65 c. c. water, 10 c. c. glacial acetic acid, 1 gm. chromic acid. Solution B: 10 c. c. 40 per cent commercial formalin, 65 c. c. water. Equal parts of A and B were mixed immediately before using.



CHROMOSOME STUDIES OF PARENTS AND  $F_1$  HYBRIDS

The somatic chromosome numbers were 42 and 14 in the wheat and rye parents and 28 in the  $F_1$  of Hybrid 128  $\times$  Rosen, as previously found by other investigators. No 16-chromosome rye plants were found. Such forms were reported by Gotoh (5) and by Belling (7) and Emme (3). Gotoh believed that occasionally a certain chromosome of the rye complement divides into two pieces, thus accounting for the extra chromosome. He reached this conclusion after finding the total linear length of all chromosomes of the 14-chromosome and 16-chromosome types to be equal. Other investigators believe that the 16-chromosome forms may originate through nondisjunction in the reduction division.

From 0 to 3 bivalents, with 28 to 22 univalents, were found in pollen mother cells in metaphases. No equatorial plate was observed in any first-metaphase cell. Usually the univalents were scattered over the entire cell. Pairing did not occur in normal fashion as in wheat and rye. Telosynaptic unions appeared to be most common, and elongated chromatin threads frequently could be seen between segregating pairs. (Fig. 1, F and I.) Orientation of the dividing bivalents sometimes was transverse to the poles of the segregating univalents. A count of cells was made to determine variation in the number of bivalents per cell. (Table 1.) Cells with two bivalents were most numerous. About 18 per cent of the cells counted had only univalent chromosomes.

TABLE 1.—Distribution of bivalent chromosomes in metaphase cells in  $F_1$  of Hybrid 128 wheat  $\times$  Rosen rye

Bivalents per cell	Cells	
Number	Number	Per cent
0.....	17	18.3
1.....	25	26.9
2.....	48	51.6
3.....	3	3.2
Total.....	93	100.0

Univalents apparently segregated at random, with frequent laggards, although what appeared to be as many as three groups of six or seven chromosomes sometimes occurred. One cell with a tripolar spindle was seen. A few cells were seen with all but one or two univalents at one pole and the remainder at the other pole.

The homotypic division was more normal, with equatorial plates more or less well developed, but the same sort of irregularity as observed in the first division was also seen. Laggards were numerous.

Tetrad formation was irregular, with micronuclei in most cells, resulting from lagging chromosomes. Microcytes were common. These usually form as a result of several chromosomes lagging together. Abnormal tetrads with as many as seven cells were seen. (Fig. 1, K-M.)

Pollen from apparently mature anthers was difficult to obtain. A few transparent pollen cells were found, varying in size from small to large. In aceto-carmin preparations pollen grains occasionally could be seen with a trace of nuclear material. Very few were seen with apparently normal nuclei.

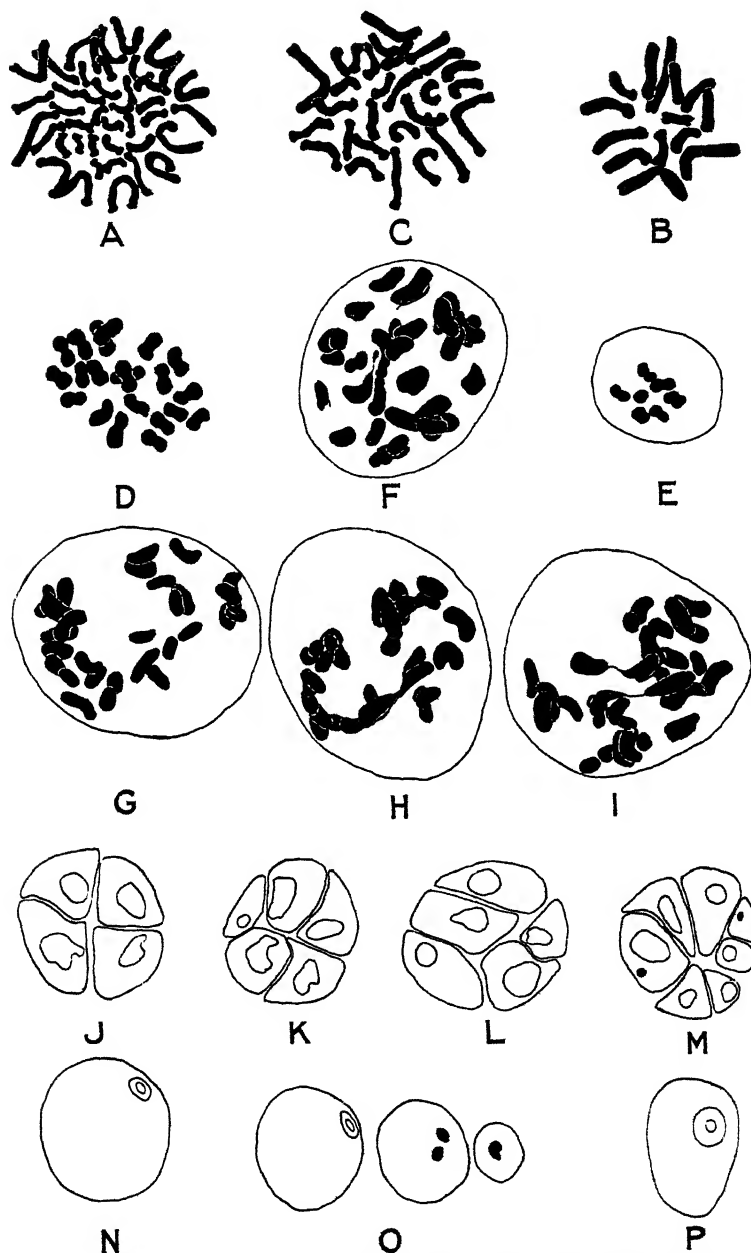


FIGURE 1.—Camera-lucida drawings of somatic and pollen mother-cell division figures from parents and  $F_1$  of Hybrid 128 × Rosen. A.—Somatic-metaphase plate of Hybrid 128 wheat ( $2n=42$ ). × 2,475. B.—Somatic-metaphase plate of  $F_1$  hybrid ( $2n=28$ ). × 2,475. C.—Somatic-metaphase plate of Rosen rye ( $2n=14$ ). × 2,475. D.—Heterotypic-metaphase plate of Hybrid 128 wheat ( $n=21$ ). × 1,700. E.—Heterotypic-metaphase plate of Rosen rye ( $n=7$ ). × 1,170. F–I.—Heterotypic-metaphase plate of  $F_1$  hybrid showing cells with all univalents (G), with two bivalents (F and H), and with three bivalents (I). End-to-end pairing best shown in F. × 1,900. J–M.— $F_1$  tetrads. Most tetrads found were 4 or 5 celled, more or less irregular, usually with one or more micronuclei; microcytes were more or less common; occasional cells were segmented as in L and M. × 1,170. N.—Hybrid 128. Outline of normal pollen grain. O.— $F_1$  hybrid. Pollen almost absent. Outline of pollen grains showing variation in size. × 1,170. P.—Rosen rye. Outline of normal pollen grain. × 1,170.

With the stains used no chromosome differences were observed in either root tip or pollen mother cells that could be used to differentiate the wheat and rye chromosomes in the hybrids. (Fig. 1.)

SOMATIC CHROMOSOME NUMBERS AND PLANT CHARACTERS OF FIRST  
BACK-CROSS FIRST-GENERATION PLANTS

Somatic chromosome numbers were obtained from root tips of nine artificially back-crossed plants and from the pollen mother-cell count of one plant. Four different back crosses were studied. The chromosome numbers are considered representative of what might be obtained from a random sample from a single hybrid. The chromosome numbers varied from 40 to 49. (Table 2.) The gametic-chromosome numbers of the original  $F_1$  therefore varied from 19 to 28, as only 21-chromosome pollen was used in back crossing. Distribution of plants by chromosome numbers was as follows: One plant each for 40, 41, 43, 44, and 45; two for 47; and three for 49. (Fig. 2, A-G.) It is probable that plants with high chromosome numbers (47 and above) are most common in the first generation after back crossing.

Plants having high chromosome numbers, 47 and above, generally were more wheatlike than those having low chromosome numbers. There was a tendency toward higher fertility in the plants having the highest chromosome numbers. The rye characters when present were reduced in intensity, as in the case of the hairy-neck character in plants 40C3, 44D1, and 44G1. (Table 2.)

The somatic-chromosome number of plant 42C1 was approximately 49. Owing to poor fixation of material it was not possible to get an exact count. This plant was the first-generation ancestor of family 98Bd3-16, a wheatlike family segregating only for kernel color, which was studied cytologically in  $F_3$ . A metaphase count could not be obtained, but cells were found which showed in side view as many as five chromosomes outside the equatorial plate. Some evidence of six or seven extra univalents in this plant was found from examination of pollen tetrads. With few exceptions, tetrad formation was regular, the cells being of about equal size, but numerous micronuclei were seen. Numbers of tetrads with micronuclei in high numbers were as follows: One with 12, two with 11, one with 8, two with 6, and one with 5. (Fig. 2, H-K.) The maximum number of micronuclei in a single cell was five, although 4 micronuclei were found in each of two cells in another tetrad. The number of univalent chromosomes making possible such numbers must have been at least six or seven. The observed root-tip count therefore must have been approximately correct.

SOMATIC AND POLLEN MOTHER-CELL STUDIES OF HYBRID 128  $\times$  ROSEN FIRST  
BACK-CROSS THIRD-GENERATION AND SECOND BACK-CROSS THIRD-GENERATION  
FAMILIES

Chromosome counts on root tips and pollen mother cells in three  $F_3$  families of Hybrid 128  $\times$  Rosen back crosses, together with descriptions of their characters as to presence or absence of hairy neck, kernel color, and average length of kernel, are presented in Table 3. Family 98Bd3-16, grown from a red-kerneled selection of family 98Bd3 (fig. 3, C), produced about equal numbers of red-kerneled and white-kerneled plants. Root-tip counts were made on 12 plants in family 98Bd3-16. All white-kerneled plants had 42 chromosomes, and the red-kerneled plants had 43 and 44 chromosomes. Spike characters are shown in Figure 4, B, C, and D.

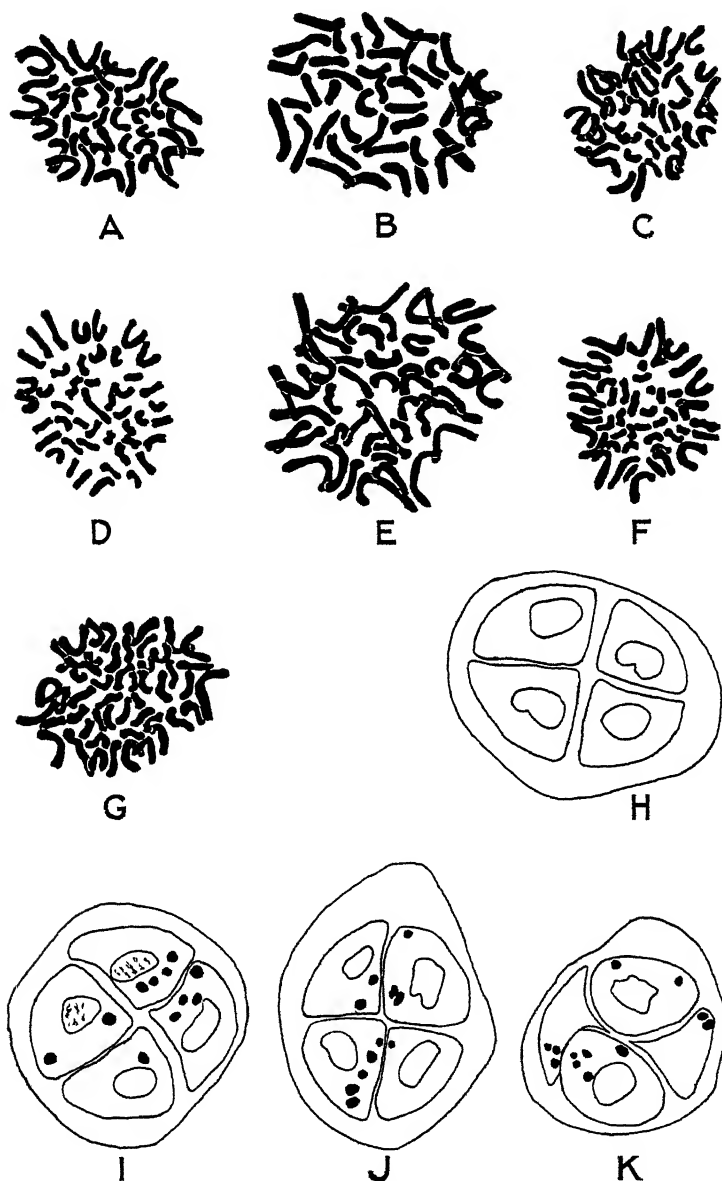


FIGURE 2.—Camera-lucida drawings of somatic chromosomes of seven first back-cross first-generation wheat × rye hybrid plants, and characteristic tetrads of plant 42C1. A.—Somatic metaphase of plant 40E1 ( $2n=40$ ).  $\times 2,000$ . B.—Somatic metaphase of plant 42 ( $2n=43$ ).  $\times 2,600$ . C.—Somatic metaphase of plant 44E1 ( $2n=44$ ).  $\times 2,000$ . D.—Somatic metaphase of plant 42B ( $2n=45$ ).  $\times 2,600$ . E.—Somatic metaphase of plant 40C8 ( $2n=47$ ).  $\times 3,150$ . F.—Somatic metaphase of plant 44D ( $2n=49$ ).  $\times 2,000$ . G.—Somatic metaphase of plant 44F ( $2n=49$ ).  $\times 2,000$ . H—K.—Tetrads of plant 42C1, a 49-chromosome plant (approximate number) showing up to 11 micronuclei, resulting from the unpaired rye chromosomes.  $\times 1,225$

TABLE 2.—Character expression and somatic chromosome numbers in first back-cross first-generation wheat × rye hybrid plants

Back-crossed generation and parent	Back-cross No.	Plant characters				Fertility of selfed plants			Kernel characters			Chromosome number
		Height	Spike length	Head type	Hairy neck	Number	Kernels	Seed set	Color	Average length	Development	
Red Winter × Rosen.		Inches	Cm.		Degree	Number	Number	Per cent		Mm.		2 n
SB <sub>2</sub> S <sub>1</sub> a	40E	50	15	Long lax.	None	78	1	1.3	Red			40
SB <sub>2</sub> S <sub>1</sub> a	40C3	55	13	do.	Light	76	0					47
Hybrid 123 × Rosen.												
WB <sub>1</sub> W <sub>1</sub>	42A1	46	8	Long club.	do.	88	10	11.4	Red		Slightly shriveled	43
WB <sub>1</sub> W <sub>1</sub>	42B1	39	5.5	Dense club.	None	84	0	0	do.	6.9		45
WB <sub>1</sub> W <sub>1</sub>	42C1	49	5.5	do.	do.	94	2	2.1	do.	7	One medium plump, one shriveled.	49
Kaured × Dakold:												
WB <sub>1</sub> W <sub>1</sub>	43A1		11	Lax.	Medium heavy	74	0					41
Kaured × Rosen:												
WB <sub>1</sub> W <sub>1</sub>	44D1	36	14.5	do.	Light	82	3	3.7	Red	7.3	Badly shriveled	49
WB <sub>1</sub> W <sub>1</sub>	44E1		6	do.	Medium heavy		0					44
WB <sub>1</sub> W <sub>1</sub>	44F1		11	do.	None		0					49
WB <sub>1</sub> W <sub>1</sub>	44G1	48	13	do.	Light at top	98	4	4.1		6.8	Slightly shriveled	47
WB <sub>1</sub> W <sub>1</sub>			5	Club.	None	71	124	1.75	White	5.1	Badly shriveled	42
Hybrid 123 ×				Lax.	do.	283	672	2.37	Red	6.9	Plump	42
Kaured			10	do.	do.	83	104	1.26	do.	8.1	Medium plump	42
Red Winter <sup>f</sup>		40	13	Long lax.	do.							

\* Average of 10 plants.

† Average of 4 plants.

e From gametic chromosome number 20-II+1-1.

d Average of 2 plants.

a S = spelt.

b Approximately.

TABLE 3.—*Character expression and somatic and gametic chromosome numbers in three back-crossed families of Hybrid 128 × Rosen, 1929*

Back-crossed generation, family, and parent plant No.	Progeny plant <sup>a</sup>	Hairy neck (degree)	Kernel color	Kernel length	Chromosome numbers	
					2n	n
WR <sub>1</sub> W <sub>1</sub> W <sub>3</sub> : 98Bd3-16.....		None.....	Red.....	Mm. 6.0		
	A-1.....	do.....	do.....	6.5	44	22 <sup>b</sup> II
	A-2.....	do.....	do.....	6.7		21 II+1 <sup>c</sup> I
	A-3.....	do.....	White.....	5.7	42	21 II
	A-4.....	do.....	do.....	6.4	42	
	A-5.....	do.....	do.....	6.5	42	
	A-6.....	do.....	Red.....	6.5	43	
	A-7.....	do.....	White.....	6.5	42	
	A-8.....	do.....	Red.....	7.0	43	21 II+1 I
	A-9.....	do.....	White.....	5.9	42	
	A-10.....	do.....	do.....	6.2	42	21 II
	A-11.....	do.....	Red.....	6.4	44	22 II
	A-12.....	do.....	White.....	6.4	42	21 II
	A-13.....	do.....	Red.....	6.4	43	21 II+1 I
WR <sub>1</sub> W <sub>3</sub> : 42A1-23.....		Heavy.....	do.....	6.6		
	B-1.....	None.....	do.....	6.7	43	21 II+1 I
	B-2.....	do.....	do.....	7.0	44	22 II
	B-3.....	do.....	do.....	6.8	44	
	B-4.....	Heavy.....	White.....	6.8	45	
	B-5.....	Medium heavy.....	Red.....	6.6	45	
WR <sub>1</sub> W <sub>3</sub> : 42A1-33.....		None.....	White.....	6.1	43	
		Medium heavy.....	do.....	7.4		
	C-1.....	Heavy.....	do.....	8.2	45	21 II+3 I
	C-2.....	None.....	do.....	6.5	42	
	C-3.....	Medium.....	do.....	6.7	45	22 II+1 I
	C-4.....	Heavy.....	do.....	6.4	42	20 II+2 I
	C-6.....	None.....	do.....	6.3	43	21 II+1 I
	C-7.....	Heavy.....	do.....	7.9	47	21 II+5 I
	C-8.....	Medium.....	do.....	7.9	44	
	C-15.....	None.....	do.....	5.8		18 II+4 I
Hybrid 128.....		do.....	do.....	6.5	42	21 II

<sup>a</sup> For convenience capital letters are substituted for the parent-plant number.<sup>b</sup> Roman numeral II indicates two paired chromosomes, which usually are seen as a double chromosome or bivalent with the members of the pair lying side by side.<sup>c</sup> Roman numeral I indicates a single unpaired chromosome or univalent.

Pollen mother-cell counts showed 21 bivalents in the white-kerneled plants, and 21 bivalents plus 1 univalent, and 22 bivalents, respectively, in the red-kerneled 43-chromosome and 44-chromosome plants.

Pairing was normal in the 42-chromosome plants, and no laggards were seen in first or second divisions. (Fig. 5, B and C.) Tetrad for mation also was regular without micronuclei, a few being observed in one plant only.

Twenty-one bivalent chromosomes and a single univalent were found in the 43-chromosome plants. (Fig. 5, E.) The univalent was found to lag and to divide in late anaphase, the divided halves going to their respective poles. Second division was normal except for the lagging members of the univalent. Tetrad formation was normal except for the occurrence of micronuclei. No micronuclei were observed in approximately 20 to 40 per cent of the tetrads examined, while from one to two micronuclei per tetrad were found in the remainder. Apparently the extra chromosome was included in the nucleus where no micronuclei occurred.

Normal pairing was found in the 44-chromosome plants. (Fig. 6, B and I.) At metaphase, however, there was a tendency for the members of the extra pair either to precede the main division or to lag. (Fig. 6, C, J, and D.) Most often the early separation took place, as micronuclei were present in only a small number of tetrads.

Somatic chromosomes were counted in root tips of six plants of  $WR_1W_3$  back-cross family 42A1-23, which was segregating for kernel color and hairy neck. In one white-kerneled and in one red-kerneled plant, both without hairy neck, the number was 43. Two red-kerneled, nonhairy necked plants had 44 chromosomes. A red-kerneled plant with medium-heavy hairy neck had 45 chromosomes, and a white-kerneled plant with heavy hairy neck also had 45 chromosomes. In the two 45-chromosome plants the rye characters—heavy hairy neck and long kernels, and medium-heavy hairy neck and red kernels—appear to have been caused by the same number of extra rye chromosomes. (See fig. 7 for spike characters.)

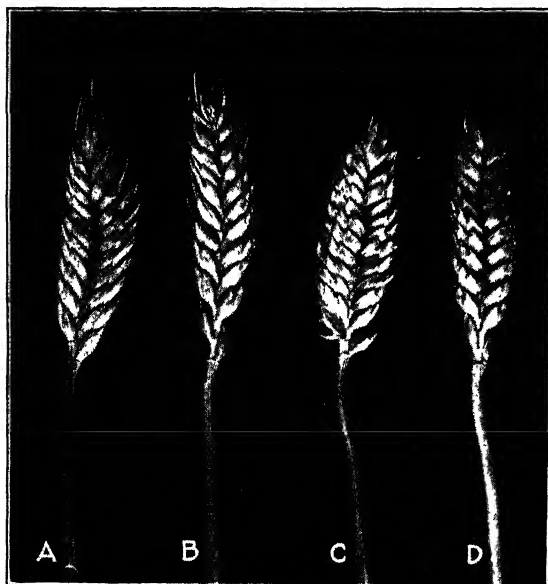


FIGURE 3.—Heads of second back-cross first-generation hybrids and parents. A,  $WR_1W_1$  parent plant, believed to have been originated from somatic gamete. Thus it had an entire somatic complement (2n) of wheat+7 rye chromosomes. When back-crossed to Hybrid 128 (D) it produced plants of type shown in B and C. C was homozygous for wheat with an extra rye chromosome, which is believed to have produced the red kernel color

Pollen mother cells were studied in only two plants of this family. Progeny plant B-1, with red kernels, had 21 bivalents plus 1 univalent, or 20 bivalents plus 3 univalents. From 2 to 3 chromosomes were seen outside the equatorial plate in side view. In homotypic division at late anaphase or early telophase three lag-gards were seen in both cells of the dividing tetrad. Tetrad formation was fairly normal, with micronuclei in about the same proportion as in the red-kerneled, 43-chromosome plants of

family 98Bd3-16. Many micronuclei were in pairs. Ten were single, 20 were double, and 2 were triple. Most pollen grains had micronuclei. It is possible that 3 chromosomes in plant B-1, 1 bivalent and 1 univalent, were rye.

A count of 22 bivalent chromosomes was made in plant B-2. (Fig. 5, J.) In a side view of the equatorial plate and in a number of other metaphase side views one chromosome which looked like a bivalent was seen in three contiguous cells. In second telophase one divided lagging chromosome was seen in each cell of the dividing dyad. Tetrad formation appeared normal, there being micronuclei in only a few cells. This is believed to be a wheat plant with an extra pair of rye chromosomes.

Somatic-chromosome numbers were counted in seven plants of  $WR_1W_3$  back-cross family 42A1-33 segregating for hairy neck and length of kernel. Two plants had 42 chromosomes, two had 45, and the remaining three had 43, 44, and 47. (See fig. 8 for spike characters.)

Both 42-chromosome plants had normal length of kernel (like parent) but one had heavy hairy neck. The latter was believed to have 20 bivalent wheat chromosomes plus 2 univalent rye chromosomes. One of the two 45-chromosome plants, C-1, had heavy hairy neck

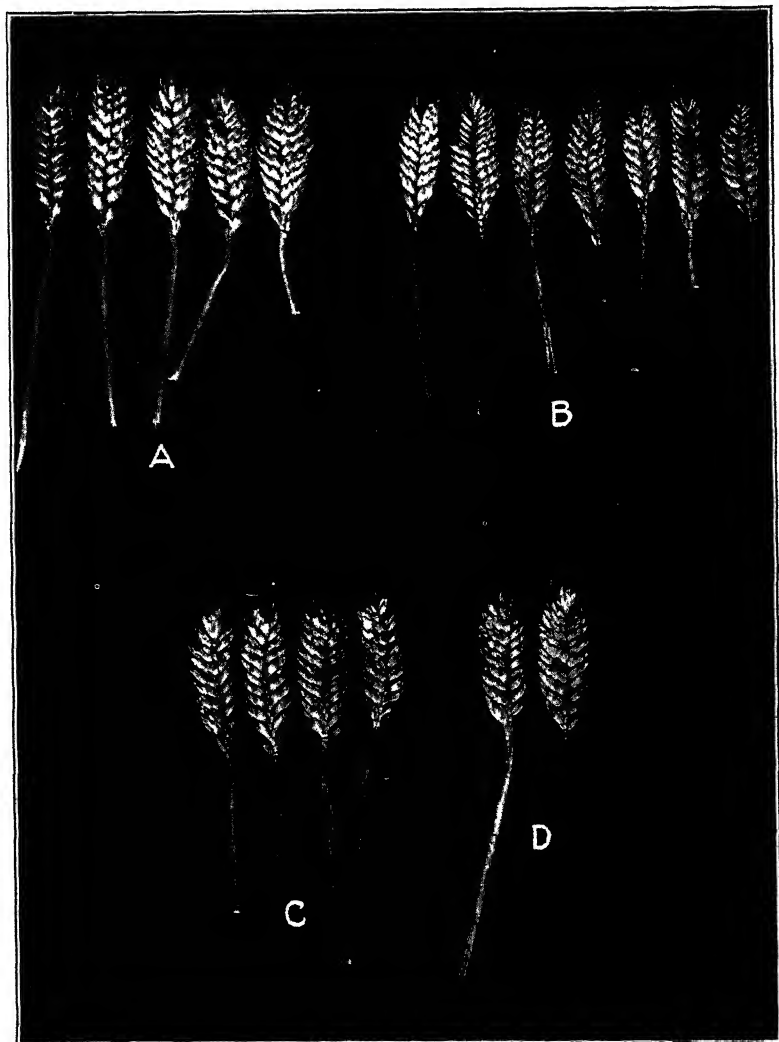


FIGURE 4.—Heads of Hybrid 128 wheat parent and of 42, 43, and 44 chromosome segregates of family 98Bd3-16. A, 5 heads from Hybrid 128 parent; B, 7 heads from 42-chromosome plants; C, 4 heads from 43-chromosome plants; D, 2 heads from 44-chromosome plants. The 42-chromosome plants were whitekerneled and in all respects identical with Hybrid 128. The 43-chromosome and 44-chromosome plants were redkerneled owing to the presence of an added univalent or a bivalent rye chromosome.

and a very long kernel (8.2 mm.). It was found to have 21 bivalents and 3 univalents. The other 45-chromosome plant, C-3, had medium-heavy hairy neck and medium-long kernel. It was found to have 22 bivalents and 1 univalent. Plant C-7, having heavy hairy neck and long kernel (7.9 mm.), had 47 chromosomes, which in meiosis showed 21 bivalents and 5 univalents.



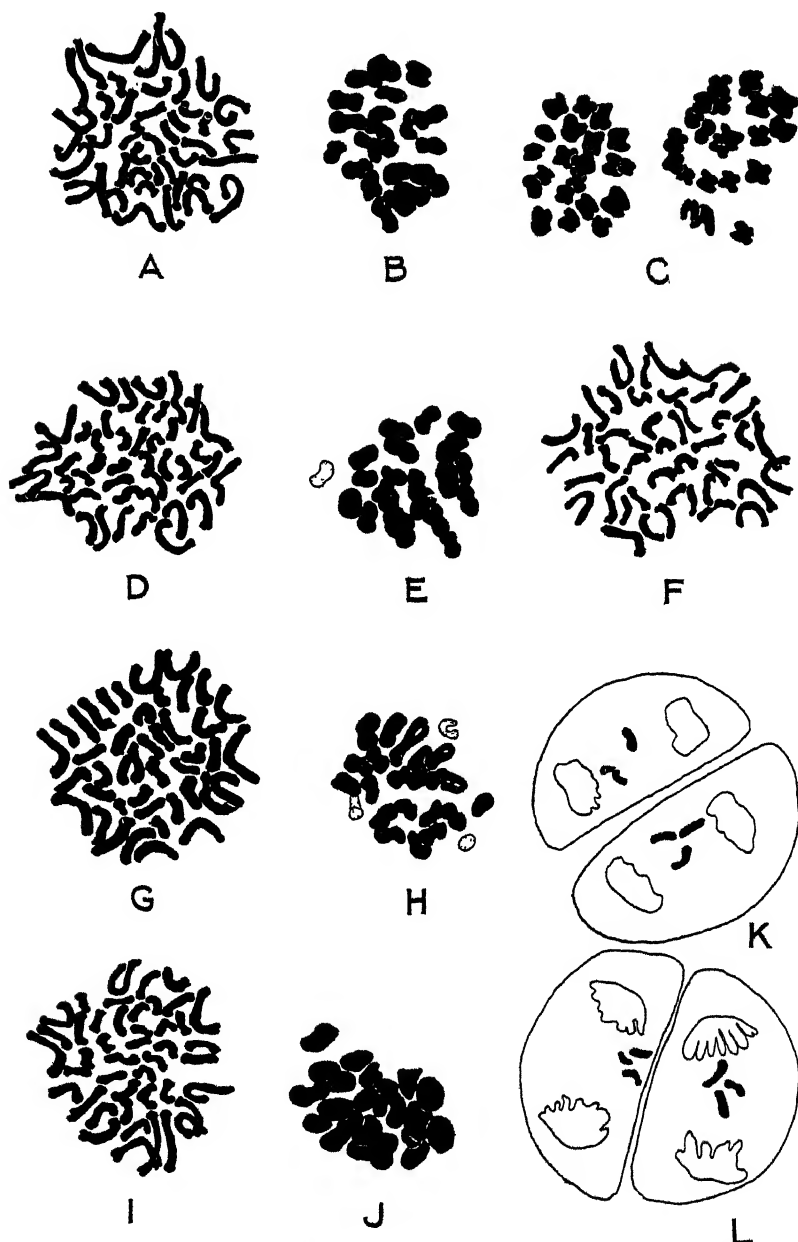


FIGURE 5.—Camera-lucida drawings of somatic and pollen mother-cell division figures of plants from second back-cross third-generation family 98Bd3-16 and from first back-cross third-generation family 42A1-23. A.—Somatic-metaphase plate from plant A-12, white-kerneled plant same as parent type ( $2n=42$ ).  $\times 2,475$ . B.—Heterotypic-metaphase plate A-12 ( $n=21$ ).  $\times 1,900$ . C.—First anaphase. No lagging, behavior regular.  $\times 1,900$ . D.—Somatic-metaphase plate from red-kerneled plant A-13 ( $2n=43$ ).  $\times 2,475$ . E.—Heterotypic-metaphase plate, A-13 ( $n=21$  II+1 I).  $\times 1,900$ . F.—Somatic-metaphase plate of B-8, a red-kerneled, hairy-necked plant ( $2n=45$ ).  $\times 2,475$ . G.—Somatic-metaphase plate of red-kerneled, nonhairy-necked plant B-1 ( $2n=43$ ).  $\times 2,475$ . H.—Heterotypic-metaphase plate of C-1 ( $n=21$  II+3 I).  $\times 1,900$ . I.—Somatic-metaphase plate of B-2, a nonhairy-necked, white-kerneled, and long-kerneled plant ( $2n=44$ ).  $\times 2,475$ . J.—Heterotypic-metaphase plate of plant B-2 ( $n=22$ ).  $\times 1,900$ . K-L.—Second telophase of plant B-2 with three lagging chromosomes in each dyad.  $\times 1,900$

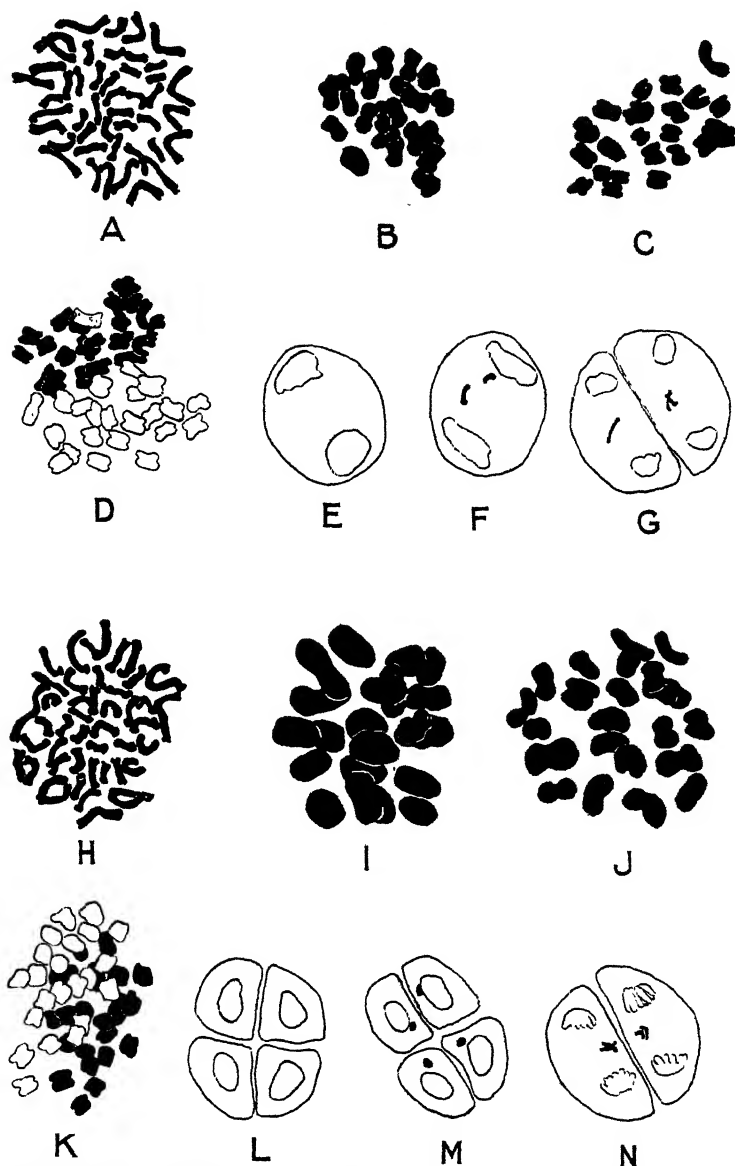


FIGURE 6.—Camera-lucida drawings of division figures from first and second back-cross third-generation hybrid plants of Hybrid 128  $\times$  Rosen, including family 98Bd3-16, segregating for kernel color only. A.—Somatic-metaphase plate of red-kerneled plant A-1 ( $2n=44$ ).  $\times 2,475$ . B.—Heterotypic-metaphase plate of A-1 ( $n=22$ ).  $\times 2,475$ . C.—Heterotypic-metaphase plate of A-1. Both members of extra pair at upper right.  $\times 2,475$ . D.—First anaphase of A-1; 21 divided chromosomes approaching the poles with the two members of the extra bivalent (stippled) at about the same level between the two groups.  $\times 2,475$ . E.—First telophase of A-1 showing clean separation. Usually no laggards.  $\times 825$ . F.—First telophase of A-1 showing members of divided extra pair. Only few cells with laggards.  $\times 825$ . G.—Second telophase with single and divided chromatid, respectively, in the two cells of the dyad.  $\times 825$ . H.—Somatic-metaphase plate of red-kerneled plant A-11 ( $2n=44$ ).  $\times 2,475$ . I.—Heterotypic-metaphase plate of A-11 ( $n=22$ ).  $\times 1,900$ . J.—Heterotypic-metaphase plate of A-11. Divided extra pair at upper right of plate.  $\times 1,900$ . K.—First anaphase with 22 chromosomes in each group.  $\times 1,900$ . L.—Tetrads of plant A-11. Mostly regular as in L, but laggards also seen in a few cells. M shows one micronucleus in each cell of the tetrad. N shows a dividing chromatid in each cell of the dyad.  $\times 825$ .

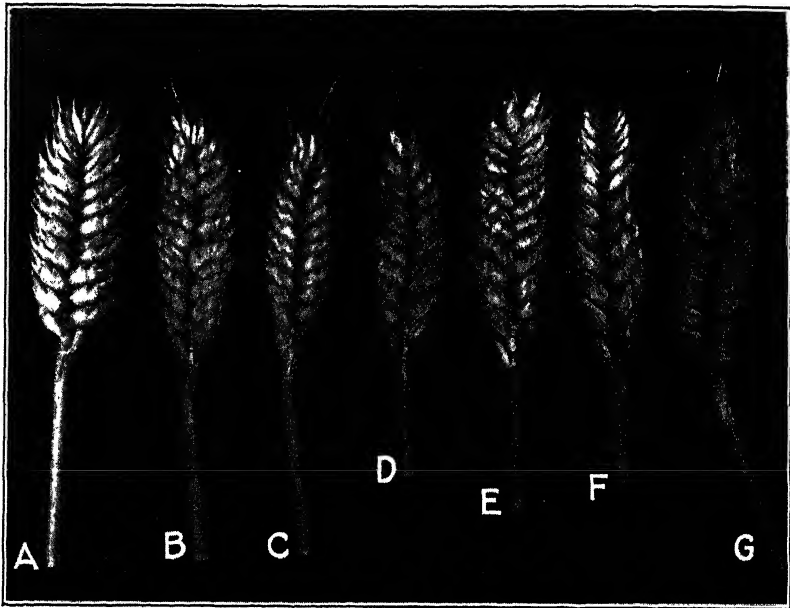


FIGURE 7.—First back-cross second-generation parent spike and third-generation segregates of Hybrid 128 X Rosen family 42A1-23 A, Parent head; B-G, segregates. Chromosome numbers of segregates: B and C, 43; D and E, 41; F and G, 45. E and F were hairy necked, the remainder, glabrous

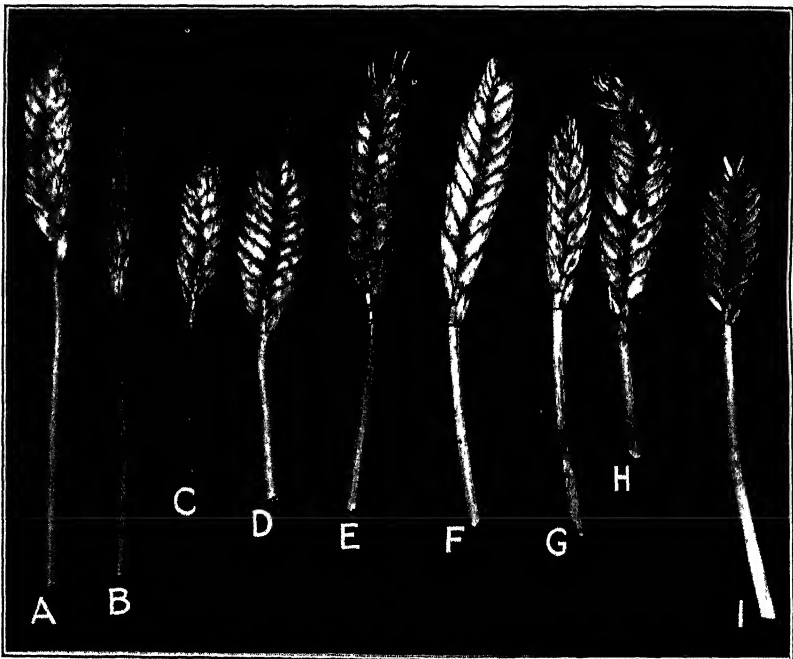


FIGURE 8.—First back-cross second-generation parent spike and third-generation progeny heads of Hybrid 128 X Rosen family 42A1-33. A, Parent head; B-I,  $WR_1W_2$  segregates. Chromosome numbers of segregates: B, 40; C and D, 42; E, 43; F, 44; G and H, 45; I, 47

Examination of pollen mother cells in seven plants of this family showed that pairing was on the whole quite normal, although unpaired chromosomes were found in all plants. Micronuclei were seen in all tetrads. Pollen grains with micronuclei frequently were observed to disintegrate.

Plant C-4, having heavy hairy neck, had 42 chromosomes, 20 of which were bivalents and 2 univalents. (Fig. 9, B.) This plant was a little below normal in stature and had reduced fertility and badly shriveled kernels. Reduction division seemed to proceed normally, however, and no lagging was observed in first anaphase, although there was some lagging in second anaphase.

In plant C-7, with 47 chromosomes, the 21 bivalents with the 5 univalents were easily distinguishable. (Fig. 9, F.) The latter were near the periphery of the metaphase group. They were more slender and usually more curved than the bivalents. A variable number of laggards were seen in the second-division figures (up to two and three) in each cell. Tetrads with micronuclei were the rule. A few tetrads with microcytes were seen. Pollen grains were variable in size. Plant C-7 was more reduced in stature than plant C-4. It had heavy hairy neck and long kernel (7.9 mm.).

Only a pollen mother-cell count was made on plant C-15. On account of scarcity of material, root-tip counts could not be obtained.

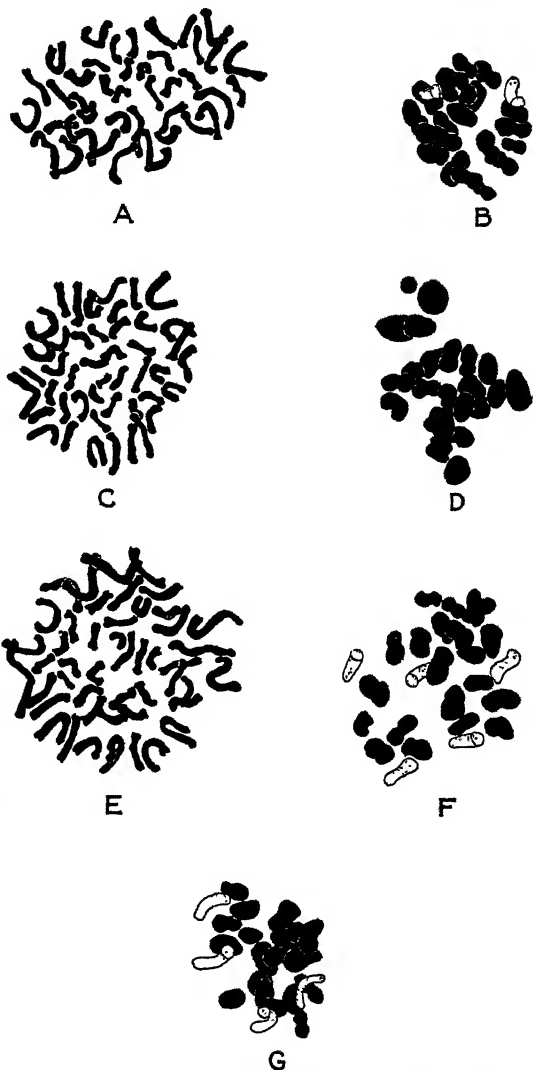


FIGURE 9.—Camera-lucida drawings of somatic and pollen mother-cell division figures of plants from first back-cross third-generation family 42A1-33, segregating for hairy neck and long kernel. A.—Somatic-metaphase plate of heavy hairy-necked, white-kernelled plant C-4 ( $2n=42$ ).  $\times 3,450$ . B.—Heterotypic-metaphase plate of C-4 ( $n=20 \text{ II}+2 \text{ I}$ ). Univalents stippled.  $\times 2,640$ . C.—Somatic-metaphase plate of hairy-necked, white-kernelled plant C-3 ( $2n=45$ ).  $\times 3,450$ . D.—Heterotypic-metaphase plate of C-3 ( $n=22 \text{ II}+1 \text{ I}$ ). Univalent stippled.  $\times 2,640$ . E.—Somatic-metaphase plate of heavy hairy-necked, long-kernelled, and white-kernelled plant C-7 ( $2n=47$ ).  $\times 3,450$ . F.—Heterotypic-metaphase plate of C-7 ( $n=21 \text{ II}+5 \text{ I}$ ). Univalents stippled.  $\times 2,640$ . G.—Heterotypic-metaphase plate of plant C-15 ( $n=18 \text{ II}+4 \text{ I}$ ). Plant reduced in stature, culms slender, neck glabrous, nearly sterile (four kernels from 15 heads).  $\times 2,640$ .

The plant was interesting because it was very much reduced in size. Its culms were slender, with slender spikes, long glumes, and no hairy neck. Sterility was complete except for a few kernels (four in 15 heads), which probably resulted from chance pollination. This plant was found to have 18 bivalents plus 4 univalents. (Fig. 9, G.) Tetrad formation proceeded quite regularly, but micronuclei were present in most cells. No mature pollen was obtained, but droughty conditions may have been partly responsible for its absence.

## MICRONUCLEI

The results of a study of the number of micronuclei found in tetrads of first and second back-cross third-generation plants are presented in Table 4. The presence of micronuclei is important, for they indicate lagging and unpaired chromosomes.

TABLE 4.—*Relationship of the number of micronuclei to the number of gametic chromosomes and to hairy neck and kernel color in first and second back-cross third-generation hybrids of Hybrid 128×Rosen, 1929*

Back-crossed generation, parent, and progeny-plant No.	Tetrads with indicated micronuclei						Total tetrads	Gametic chromosomes	Hairy neck	Kernel color
	None	One in one cell only	One in each of 2 cells	One in each of 3 cells	One in all 4 cells	Variable				
WR <sub>1</sub> W <sub>1</sub> W <sub>3</sub> : 98Bd3-16—	Number	Number	Number	Number	Number	Number	Number	Number and kind		
A-2.....	6	14	14				34	21 II +1 I.	None.....	Red.
A-6.....	17	16	16				49	* 43.....	do.....	Do.
A-8.....	9	5	7				21	21 II +1 I.	do.....	Do.
A-13.....	18	34	27			1	80	21 II +1 I.	do.....	Do.
A-1.....	50	2		4	2		58	22 II.....	do.....	Do.
A-11.....	13	5	1	3		1	23	22 II.....	do.....	Do.
A-3.....	89						89	21 II.....	do.....	White.
A-5.....	51						51	* 42.....	do.....	Do.
A-10.....	40						40	21 II.....	do.....	Do.
A-12.....	58		2				60	21 II.....	do.....	Do.
WR <sub>1</sub> W <sub>3</sub> : 42A1-23—										
B-1.....	11	20	22	1	1		68	21 II +1 I.	do.....	Red.
B-2.....	22	1	1	1			25	22 II.....	do.....	White.
WR <sub>1</sub> W <sub>3</sub> : 42A1-33—										
C-3.....	8	16	6	1		1	31	22 II +1 I.	Medium heavy	Do.
C-4.....	17	13	4				34	20 II +2 I.	Heavy.....	Do.
C-6.....	36	26	4	2			68	21 II +1 I.	None.....	Do.
C-7.....	5	2	3				10	21 II +5 I.	Heavy.....	Do.
C-15.....	18	41	33	1			93	18 II +1 I.	None.....	Do.
Hybrid 128.....	( <sup>b</sup> )							21 II.....	do.....	Do.

\* Somatic-chromosome numbers.

<sup>b</sup> No micronuclei observed.

In family 98Bd3-16, segregating for kernel color only, the white-kerneled plants showed no micronuclei, as division proceeded normally. The red-kerneled, 43-chromosome plants had from one to two micronuclei in one or more cells of most tetrads, owing to the lagging univalent. The 44-chromosome plants with 22 bivalents were without micronuclei in most tetrads. A few tetrads had a single micronucleus in each of the four cells. Some pollen sterility would be expected in such plants.

The conditions as to micronuclei in 22-bivalent-chromosome plant B-2 (family 42A1-23) were similar to those in the 22-bivalent-chromosome plants described in the preceding paragraph. Micronuclei were present in varying numbers in all the remaining plants of family 42A1-23.

## DEVELOPMENT OF POLLEN GRAINS

A study of pollen grains was made on the  $WR_1W_3$  and  $WR_1W_1W_3$  plants whose chromosome number had been determined, and on  $F_1$  hybrid plants. Data on chromosome number, presence or absence of hairy neck, kernel color, and length of kernel are shown in Table 5. Counts were made on 7 plants in family 98Bd3-16, on 2 plants in family 42A1-23, and on 5 plants in family 42A1-33.

Apparently normal pollen-grain development occurred in the 42-chromosome white-kerneled plants of family 98Bd3-16. This family, as already stated, was segregating for kernel color only. From 95 to 98 per cent of normally nucleated pollen grains were found. Sixty-five and 56 per cent of normally nucleated pollen grains were found in the red-kerneled plants A-1 and A-11, respectively, each having 22 bivalent chromosomes.

The presence of the rye chromosomes probably was responsible for part of the poor pollen development. Similar results were obtained from the red-kerneled 43-chromosome plants.

The percentages of normal pollen found in families 42A1-23 and 42A1-33 varied from low to medium. On the average the percentage of apparently normal pollen varied with the number of univalents present. Plant B-1, however, with a chromosome number of 43, of which 1 bivalent and 1 univalent are believed to have been rye, produced only 16 per cent of normal pollen. Plant B-2, with 22 bivalent, 1 of which is believed to have been rye, also produced a low percentage of normal pollen, although slightly higher than that of plant B-1.

The presence of rye chromosomes seemed to interfere with normal pollen development, but dry, hot weather at blossoming time also may have been partly responsible for the high degree of sterility, which was found even in some plants of the parent Hybrid 128. About 99 per cent of normal pollen was found in most plants of the parent Hybrid 128, and 69 per cent in the parent Rosen. Only 1 nucleated pollen grain among 413 was found in the  $F_1$  of Hybrid  $128 \times$  Rosen.

## DISCUSSION

GAMETE FORMATION IN  $F_1$ 

Hybrids between wheat and rye have been shown to be completely self-sterile. When such  $F_1$  hybrids are back crossed to wheat a low percentage of fertility is obtained. The hybrid gametes responsible for this limited fertility appear to originate in two ways: (1) By random distribution of unpaired chromosomes; and (2) by the formation of somatic gametes. The occurrence of the first type is common where univalent chromosomes are found and has been demonstrated by numerous investigators of various plant hybrids. The occurrence of somatic gametes in plants (*Hieracium* sp.) was demonstrated by Rosenberg (12). He found that the heterotypic division was interrupted at about late prophase or metaphase and a new nucleus reconstituted with the diploid number of chromosomes.

The chromosome numbers in ovules of the  $F_1$  hybrid were determined by back crossing to the wheat parent. Somatic-chromosome numbers varied from 40 to 49 in 10 progeny plants. The chromosome number of the pollen gamete was 21. Therefore, the chromosome

TABLE 5.—*Pollen grains, gametic chromosomes, hairy neck, kernel color, and kernel length in first and second back-cross third-generation hybrids of Hybrid 128 × Rosen, 1929*

Back-crossed generation, parent, and progeny- plant No.	Pollen grains					Remarks	(gametic chromo- somes	Hairy neck	Kernel color	Kernel length
	Nucle- ated with micro- nuclei	Empty	With normal nuclei only							
			Number	Per cent						
WR <sub>1</sub> W <sub>2</sub> W <sub>3</sub> 95B13-16—	Number	Number	Number	Per cent		Number and kernel				Min.
A-2	80	270	77		Mostly normal	21 II+1 I	None	Red		7
A-8	6	141	55		do.	21 II+1 I	do.	do.		7
A-1	108	153	65		Size uniform, round	22 II	do.	do.		6
A-11	44	56	56		do.	22 II	do.	do.		6
A-3	21	634	97		Normal, uniform, some disintegrating	21 II	do.	White		6.4
A-10	5	230	98		do.	21 II	do.	do.		7
A-12	3	230	95		Normal, well developed	21 II	do.	do.		6.4
WR <sub>1</sub> W <sub>2</sub> W <sub>3</sub> 42A1-23—										
B-1	188	14	16		Normal size but mostly micronucleated	21 II+1 I	do.	Red		6.7
B-2	87	7	39		do.	22 II	do.	White		6
WR <sub>1</sub> W <sub>2</sub> W <sub>3</sub> 42A1-33—										
C-1	64	13	157		Size variable	21 II+3 I	Heavy	do.		8.2
C-3	61	28	200		Size uniform	22 II+1 I	Medium heavy	do.		6.7
C-4	112	41	113		Some with micronuclei disintegrating	20 II+2 I	Heavy	do.		6.1
C-6	61	4	133		Size uniform	21 II+1 I	None	White		6.4
C-7	2	53	45		Size variable	21 II+5 I	Heavy	do.		7.3
Hybrid 128	3	217	99		Size uniform, round	21 II	None	do.		6.3
Rosen	115	261	69		Size uniform, oval	7 II	Heavy	do.		6.5
Hybrid 128×Rosen	412	1	2		Size very large to small Pollen grains transparent, with nucleated portion in occasional cells.	(c)	Medium heavy	Pale red to gray.		

\* 28 to 22 univalents+0 to 3 bivalents.

numbers in the ovules must have varied from 19 to 28. The parent ovules of three of these plants had a gametic-chromosome number of 28, and two had 26. The chances of obtaining these numbers with random distribution in the  $F_1$  gametes are negligibly small. Assuming random distribution and equal viability of gametes, the chances were calculated by expanding the binomial  $(\frac{1}{2} + \frac{1}{2})^n$ , where  $n$  is the chromosome number of the gamete. Therefore it is seen that the chance of securing 28-chromosome gametes by random distribution is 1 in about 278,000,000, and the chance of securing 26-chromosome gametes is 1 in 718,000. The fact that one-half of the plants examined originated from 28-chromosome and 26-chromosome ovules seems definitely to exclude random assortment as the only mode of gametic-chromosome distribution.

A small number of viable gametes with chromosome numbers varying from 19 to 22, is entirely possible, for from about 0.1 to 2.5 per cent of such gametes should occur, on the basis of random assortment. Twenty-three-chromosome gametes should occur once in about 2,800 times. The chance of securing gametes with lower chromosome numbers is limited by their ability to form viable combinations. If all or nearly all rye chromosomes come together in a single gamete the latter probably will be viable if fertilized with rye pollen.

The lower and upper limits of gametic-chromosome numbers in somatic gametes in  $F_1$  wheat × rye hybrids can only be surmised. Rosenberg (13) noted irregularities in chromosome distribution in somatic gametes. Karpetschenko (6), in crosses of *Raphanus* ( $n = 9$ ) × *Brassica* ( $n = 9$ ), found hybrids with triploid, tetraploid, and other polyploid combinations. Plants were found with chromosome numbers varying slightly from exact polyploid numbers as follows: A hypertriploid, 28–29; a hypertetraploid, 37–38; a hypopentaploid, 40–42; a hypoheptaploid, 51–53; a hypoenneaploid, 78. The somatic-chromosome number of  $WR_1W_1$  back-crossed plant 42C1 was counted as 51, but scarcity of material made it impossible to determine definitely this number, and the plant is listed as having approximately 49 chromosomes. Wheat-rye somatic gametes with chromosome numbers above 28 are not beyond possibility. It is interesting to note that the gametic-chromosome numbers 24, 26, and 28 were found, but not 25 or 27. This would seem to indicate that in these cases entire pairs of chromosomes had been eliminated in gametic formation. The limited pairing in the  $F_1$  hybrids would effect changes in the calculated proportion of gametic-chromosome numbers. This probably also hinders somatic-gamete formation in that the dividing pairs initiate early cell formation and chromosome segregation.

The occurrence of somatic gametes in wheat × rye hybrids may have possibilities for amphidiploid combination (both chromosome complements doubled) of wheat and rye. Amphidiploids have been produced by several investigators in a variety of plants. An example of such forms in cereals is given by Tschermak and Bleier (16) in their *Aegilotriticum* hybrids ( $n = 28$ ), from *Aegilops* ( $n = 14$ ) × *Triticum dicoccoides* ( $n = 14$ ) and *T. dicoccum* ( $n = 14$ ).

#### STERILITY RELATIONS

The slightly fertile wheat × rye hybrids and back crosses, in successive generations of selfing, increase their fertility with the elimination of rye chromosomes and the return of gametes to the parent wheat type. Fertility appears to vary in strains with different rye characters.



High or nearly complete sterility in the selfed first-generation back-cross plants may result from nonviable pollen and egg cells, or from lethal zygotic combinations, or both. That a low percentage of viable ovules is formed is shown where such plants have been back crossed. The chief cause of sterility in the back-crossed plants from somatic gametes is the presence of extra rye chromosomes which in some way interfere with development. As the number of extra rye chromosomes is reduced the fertility of the plant increases, until with a single extra chromosome or pair of chromosomes fairly high fertility is attained.

The chromosome containing the gene or genes for red kernel color appeared to reduce fertility more than did the chromosomes containing the gene or genes for hairy neck. The chromosome causing hairy neck affects the maternal plants, while the chromosome causing red kernel affects endosperm development and thus, directly, fertility. Chromosomes producing long kernel also reduced fertility.

Extent of hairy neck was found to vary in its relation to fertility. Heavy hairy-necked plants generally were highly sterile, while light hairy-necked plants were decidedly less sterile. Hairy-necked plants appeared to be less sterile in Kanred crosses than in Hybrid 128 crosses. Cytological evidence indicates that two chromosomes or two chromosome pairs were present in the heavy hairy-necked plants. The chromosomes controlling both hairy neck and red kernel color caused a lengthening of the kernel. However, there must be other chromosomes that cause lengthening of the kernel, because long kernels were found in plants that had neither red kernels nor hairy neck.

The factors operating to produce sterility may cause striking changes in plant characters. Frequently there is reduction in height of plant, number of culms, and general vigor. Occasional dwarf plants may appear. More or less badly shriveled kernels are a reasonably certain index of abnormal chromosome relations. Sax (14) found in crosses between *Triticum durum* ( $n=14$ ) and *T. vulgare* ( $n=21$ ) that the weight (endosperm development) of  $P_1$  seeds was about one-half of the mean of the parent seeds. In  $F_2$  endosperm ( $F_1$  plant) the development of seeds varied from plump to shriveled. Sax concluded that small wrinkled grains are characteristic of partially sterile hybrids. Watkins (17) found that in crosses between 14-chromosome and 28-chromosome wheats germination and development of grain are determined largely by the chromosome constitution of the endosperm. A high percentage of germination occurs if the *vulgare* chromosomes are all diploid or triploid in the endosperm.

#### CONSTANT FORMS WITH RYE CHARACTERS

From cytologic and genetic evidence it appears possible to add a pair of rye chromosomes to the wheat complement in a more or less constant combination. The best evidence for this is found in the red-kerneled family 98Bd3-16 of Hybrid 128  $\times$  Rosen. This family was found to be pure for all wheat characters except kernel color. The red color was found to be due to the presence of either one or two extra chromosomes. Two 44-chromosome plants produced 22 bivalents in the first pollen mother-cell metaphase. The distribution of chromosomes was nearly normal, although there was some irregularity owing to laggards. Tetrad and pollen formation was fairly normal. Judging by general behavior these plants may breed true in succeeding generations.

Supplementary genetic evidence on the production of a red-kerneled strain may be adduced from family 42A1-37, which was red kerneled in 1929 except for one white-kerneled plant (4). This family was high in sterility but segregated for hairy neck and long kernels. The presence of the white-kerneled plant may be explained by the occasional elimination from both pollen and egg cells of the extra chromosome bearing the factor for red color. The union of such gametes would produce a white-kerneled plant.

In 1928 several red-kerneled plants were found which had white kernels also. These varied in number from a few to about one-fourth of all kernels of the plant. The appearance of such plants indicated that somatic mutation must have occurred at an early stage of development of the young plant, causing elimination of the chromosome determining red kernel color. As a result, white kernels were produced in a whole section of the spike.

A strain was obtained which was true breeding for hairy neck and still segregating for other rye characters. It is believed that a hairy-necked wheatlike strain can be produced if chromosomes bearing other rye characters are eliminated.

#### SUMMARY

Chromosome counts from root tips of Hybrid 128 wheat, Rosen rye, and their  $F_1$  hybrids were 42, 14, and 28, respectively. From 0 to 3 bivalents and from 28 to 22 univalents were found in  $F_1$  pollen mother cells. Cells with two bivalents apparently predominated.

Back crossing with the wheat parent showed that a small number of viable gametes are formed. Somatic-chromosome number in 10 first back-cross first-generation ( $WR_1W_1$ ) plants (9 from root tips) varied from 40 to 49. The gametic-chromosome numbers of the  $F_1$  ovules, therefore, varied from 19 to 28. The gametes with high chromosome numbers (28 and 26) must have been formed as somatic gametes, those with lower numbers (19 to 21 or 22) probably by random assortment.

Back-crossed plants from somatic gametes usually are more wheatlike and fertile than plants from gametes resulting from random assortment.

Somatic-chromosome counts were determined from root tips in 25 first back-cross third-generation ( $WR_1W_3$ ) and second back-cross third-generation ( $WR_1W_1W_3$ ) plants. Chromosome numbers varied from 42 to 47.

Chromosome counts on plants of a second back-cross third-generation family of Hybrid 128  $\times$  Rosen, segregating only for kernel color, were 42, 43, and 44. Red-kerneled plants had 43 and 44 chromosomes, and white-kerneled plants 42 chromosomes. Twenty-two bivalents were found in pollen mother cells of two 44-chromosome plants, whose subsequent behavior was almost normal. In the white-kerneled plants chromosome behavior was normal.

Two hairy-neck families, one segregating for color of kernel and the other for length of kernel, had somatic-chromosome numbers varying from 43 to 45 in the first family and 42 to 47 in the second family. All plants had from 1 to 5 univalents, except one which had 22 bivalents and whose behavior was fairly normal. Tetrad and pollen formation was abnormal in all.

Cytologic and genetic behavior indicates that by back crossing wheat  $\times$  rye hybrids with wheat it may be possible to produce constant wheatlike strains possessing rye characters. Rye factors apparently may be added to the wheat complement as whole rye chromosomes.

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# THE EFFECT OF CALCIUM FLUORIDE AND PHOSPHATE ROCK ON THE CALCIUM RETENTION OF YOUNG GROWING PIGS<sup>1</sup>

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## THE WORK OF OTHER INVESTIGATORS

The experiments of Forbes and his associates (5)<sup>2</sup> at the Ohio Agricultural Experiment Station defined in a very complete fashion the relative efficiency of several mineral compounds as calcium supplements in swine nutrition. The minerals tested included limestone floats, precipitated bone flour, special steamed bone flour, rock phosphate floats, and precipitated calcium carbonate. As compared with a nonsupplemented ration, the bone and carbonate preparations did not differ greatly in their ability to increase retention of calcium and to induce the development of strong, dense bone. The rock phosphate, however, was distinctly inferior in these respects, for in hardness, density, and breaking strength, no differences were observed between the bones of pigs that received no minerals and those that received the rock phosphate supplement. The average ash content of the humeri of the pigs fed the various mineral supplements, expressed in grams of ash per cubic centimeter of volume, was as follows: Pigs fed calcium carbonate, 0.5148 gm.; steamed bone flour, 0.5125 gm.; precipitated bone flour, 0.4786 gm.; rock phosphate floats, 0.4459 gm.; no mineral supplement, 0.3756 gm.; control, killed at the beginning of the experiment, 0.4645 gm. The rock phosphate value is distinctly lower than the value for any of the other supplements fed. All the minerals used were fed in daily amounts equivalent to 5 gm. of calcium.

Reed and Huffman (10, 11) reported that when raw rock phosphate was fed to dairy cattle to the extent of 1.5 per cent or more of the grain mixture the animals went off feed, remained below normal weight, and their teeth became very soft and in some instances wore down to the gums. The mandibles of these cattle were much thicker and the surfaces were rougher than those of normal cattle. Buckner, Martin, and Peter (3, 4) found phosphate unsatisfactory as a mineral supplement for laying hens. Hens that received rock phosphate *ad libitum* were affected with diarrhea and their egg production was impaired; the lot that received no minerals laid 60 per cent more eggs than those that received rock phosphate. Further evidence of the low value of rock phosphate as compared to bone meal is furnished by Tolle and Maynard (17), who found that white rats fed rock phosphate in quantities equal to 1.8 per cent of the ration gained in weight more slowly than rats fed bone meal. The teeth of the rats that received rock phosphate showed the changes first observed by McCollum, Simmonds, Becker, and Bunting (7) as being due to fluorine in the diet. The

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Reference is made by number (italic) to Literature Cited, p. 372.

average ash content of the bones of the rats fed rock phosphate was 10 per cent less than that of the rats fed bone meal. Phosphate rock also lowered the food consumption and checked the growth of rats fed by Sollmann, Schettler, and Wetzel (15).

E. B. Forbes was the first to suggest that the fluorine present in rock phosphate is the cause of its deleterious effects. By the method of Reynolds, Ross, and Jacob (12), which was devised especially for the analysis of phosphorite minerals, Jacob and Reynolds (6) found brown rock phosphate of Tennessee origin to contain from 2.62 per cent fluorine in "run-of-mine material" to 4.08 per cent in carefully selected lump rock. A "fertilizer grade" of the same rock contained 3.72 per cent fluorine, while a high-grade washed and ground rock contained 3.89 per cent. The present writers, following the method of Jacob and Reynolds, found 3.83 per cent fluorine in a fertilizer grade of washed raw rock phosphate of Tennessee origin.

The toxicity of fluorine to animals has been frequently observed, and even at levels which might be attained in livestock feeding by the use of rock phosphate as a calcium supplement deleterious effects have occasionally been noted. The results of studies carried on at the Illinois station (9, p. 594) indicate that for growing pigs the daily requirement of calcium is about 1.95 gm. at 30 pounds in weight, 2.10 gm. at 50 pounds, 2.25 gm. at 100 pounds, 2.40 gm. at 150 pounds, 2.55 gm. at 200 pounds, and 2.70 gm. at 250 pounds. Assuming a utilization of only 50 per cent of the dietary calcium (5) it appears that 5 gm. of calcium per day should be sufficient to meet the need of the normal growing pig for this element. The presence of as much as 3.8 per cent of fluorine in raw rock phosphate would necessitate the addition of about 0.60 gm. of fluorine to the daily ration (equal to 0.02 per cent or more of the daily food intake) to supply the entire requirement of 5 gm. of calcium. For a pig weighing 50 kgm. (110 pounds) it is apparent that the daily fluorine intake on this basis would amount to about 0.012 gm. per kilogram of body weight. Toxic effects have occasionally been observed to follow the feeding of sodium fluoride in equivalent fluorine doses; i. e., 0.027 gm. per kilogram of body weight daily.

Brandl and Tappeiner (2) report the results of feeding a dog from about 0.04 to 0.08 gm. of sodium fluoride per kilogram of body weight per day. At the higher levels of fluoride feeding diarrhea and nausea occurred. Rats fed by Sollmann, Schettler, and Wetzel (15) received daily doses of 0.00015 to 0.15 gm. of sodium fluoride per kilogram of body weight; at levels of 0.015 to 0.020 gm. no evidence of harmful effects was observed. McCollum, Simmonds, Becker, and Bunting (7) fed 0.05 per cent of sodium fluoride (0.0226 per cent of fluorine) to rats in an otherwise normal ration, and found abnormal tooth development and slightly stunted growth. Schulz and Lamb (13) fed a ration containing 0.0226 per cent fluorine to rats, with no effects noted, though at higher levels the fluorine appeared to have had a deleterious effect on growth and reproduction. Taylor (16) found that when a dairy cow was fed about 0.006 gm. of fluorine daily per kilogram of body weight (assuming a body weight of 500 kgm.) as calcium fluosilicate there eventually resulted a serious disturbance of the appetite and general health and an injurious effect on the teeth; the effects in every way duplicating those obtained by Reed and

Huffman from the feeding of rock phosphate to dairy cows. Schwyzer (14) fed rabbits 0.03 gm. of sodium fluoride daily for 10 to 20 days and observed that "some of them lost their appetite and became very emaciated."

The experiments of Bethke, Kick, Edgington, and Wilder (1) show that fluorine in the form of sodium fluoride when added to the ration of growing swine may lower the food consumption, retard the gains in weight, and modify the composition of bone with respect to its fluorine content. The fluorine content of the bones was uniformly increased as the fluorine in the ration increased. Also the breaking strength of the bones was low for those bones that contained the most fluorine. The results of analyses of the bones for ash and for the calcium, magnesium, and phosphorus content of the ash, however, show no significant differences which may be related to the fluorine intake. The deleterious effects upon food consumption, gains, and strength of bone were observed to follow the feeding of 0.038 gm. of fluorine daily per kilogram of body weight, but not the feeding of 0.021 gm.

#### PLAN OF THE EXPERIMENT

In the experiment reported in this paper, the effects of rock phosphate at different levels of intake on the calcium metabolism of growing pigs was compared with the effects of synthetic mixtures of tricalcium phosphate and calcium fluoride containing equal amounts of fluorine. Five young Duroc Jersey-Poland China crossbred pigs averaging about 155 pounds in weight at the start were used in the experiment and were confined in metabolism crates for the entire duration of the test. Their basal ration consisted of 79.5 per cent of ground corn, 15 per cent of soy bean-oil meal, 4 per cent of linseed-oil meal, 1 per cent of cod-liver oil, and 0.5 per cent of salt. The ration was mixed fresh every week or 10 days and was kept tightly covered to prevent spoilage and loss of moisture. A sample was taken from each mixing for analysis for calcium. The pigs were given distilled water to drink and a record was kept of the amount consumed. They were weighed every seven days.

There was added to the daily ration of each pig, 5 gm. of calcium in one of the following forms: (1) Pure tricalcium phosphate, (2) tricalcium phosphate and calcium fluoride combined in different proportions for different pigs, and (3) tricalcium phosphate and rock phosphate in different proportions. Each of the five pigs received a different concentration of fluorine in its mineral mixture, i. e., approximately 0.5, 1, 2, 3, and 3.8 per cent, respectively, but in each case the daily calcium addition was the same, i. e., 5 gm. During the periods in which no fluorine was added to the ration, each pig received daily 5 gm. of calcium as pure tricalcium phosphate. In Table 1 are given the kinds and amounts of the daily mineral additions for each pig in each 2-week period.

TABLE 1.—Daily mineral additions<sup>a</sup> furnishing 5 gm. of calcium added to the basal ration of each pig

Period Nos	Pig 6-9			Pig 24-3			Pig 39-30			Pig 32-30			Pig 6-6		
	$\text{Ca}_3(\text{PO}_4)_2$			$\text{Ca}_3(\text{PO}_4)_2$			$\text{Ca}_3(\text{PO}_4)_2$			$\text{Ca}_3(\text{PO}_4)_2$			$\text{Ca}_3(\text{PO}_4)_2$		
	$\text{Ca}_3$	$\text{CaF}_2$	F in mineral	$\text{Ca}_3$	$\text{CaF}_2$	F in mineral	$\text{Ca}_3$	$\text{CaF}_2$	F in mineral	$\text{Ca}_3$	$\text{CaF}_2$	F in mineral	$\text{Ca}_3$	$\text{CaF}_2$	F in mineral
1 and 2.....	Gm	Gm.	P. ct.	Gm	Gm	P. ct.	Gm	Gm.	P. ct.	Gm	Gm.	P. ct.	Gm.	Gm	P. ct.
3 and 4.....	12 90	0	12 90	12 90	0	12 90	12 90	0	12 90	12 90	0	12 90	12 90	0	12 90
5 and 6.....	12 73	0 13	0 47	12 55	0 26	95	12 21	0 52	1 90	11 87	0 77	2 84	11 60	0 98	3 62
7 and 8.....	12 90	0	12 90	12 90	0	12 90	12 90	0	12 90	12 90	0	12 90	12 90	0	12 90
9 and 10.....	12 73	13	50	12 55	27	1 00	12 21	55	2 00	11 87	82	3 00	11 60	1 03	3 83
11 and 12.....	12 90	0	12 90	12 90	0	12 90	12 90	0	12 90	12 90	0	12 90	12 90	0	12 90
13 and 14.....	11 40	1 69	50	9 83	1 37	1 00	8 56	1 7	2 00	3 07	1 12	3 00	14 60	3 83	0
	12 90	0	12 90	12 90	0	12 90	12 90	0	12 90	12 90	0	12 90	12 90	0	12 90

<sup>a</sup> The quantities of  $\text{Ca}_3(\text{PO}_4)_2$  were calculated on the assumption that the  $\text{Ca}_3(\text{PO}_4)_2$  was 100 per cent pure and would thus contain 38.739 per cent of Ca. It was found later by analysis to contain but 33.607 per cent of Ca and this percentage value was used in determining the calcium intake of the pigs. The calculations for the amounts of  $\text{CaF}_2$  fed in periods 3 and 4 are based on a content of 51.325 per cent of Ca and 48.675 per cent of F (100 per cent pure  $\text{CaF}_2$ ). For periods 7 and 8 the basis of calculation is 48.763 per cent Ca and 46.254 per cent F. An analysis of the  $\text{CaF}_2$  for Ca showed it to be but 95.018 per cent pure, containing 48.763 per cent Ca and, by calculation, 46.254 per cent of F. These last two values for Ca and F were used for determining the Ca and F intake for all periods in which  $\text{CaF}_2$  was fed. The rock phosphate was found to contain 34.233 per cent of Ca and 3.530 per cent of F.

<sup>b</sup> Rock phosphate.

The calcium balances were determined for consecutive 7-day periods. For each pig there was a fore period of two weeks without fluorine at the beginning of the experiment followed by two weeks of fluorine feeding, and then by alternate 2-week periods of no fluorine and fluorine mineral additions.

The metabolism crates in which the pigs were kept during the experiment were cleaned daily, and the feces and urine for a 24-hour period were collected and weighed. The feces were placed at once in a drying oven in a pan with the collections of the preceding days of the period, and the total feces for the period were dried in the oven to constant weight, ground to a fine powder, and kept in air-tight jars at freezing temperature until analyzed.

After the feces and bits of refused feed were removed from the screen, the screen and bottom of each crate were washed thoroughly with approximately 3 liters of boiling distilled water. These washings were added to the day's urine collection, the total filtered through a 100-mesh sieve and thoroughly mixed. A one-tenth aliquot by weight was taken and preserved by adding hydrochloric acid to approximately 0.41 per cent by weight. The combined aliquots were kept in tightly stoppered bottles at freezing temperature until analysis was completed.

Any feed remaining in the feed boxes or spilled in the crates was weighed and considered in calculating the balances.

The percentages of calcium in the feed samples and in the feces were determined by McCrudden's method (8), the calcium oxalate precipitate being titrated with standard  $\text{KMnO}_4$ . McCrudden's method was followed in the analysis of urine; in this case the calcium oxalate was ignited and weighed as calcium oxide.

## THE FOOD CONSUMPTION AND GAIN OF THE PIGS

During the last two or three weeks of the experiment all of the pigs showed a tendency to eat less. Several times they vomited, but this could not be attributed to the fluorine content of the ration, since it occurred during periods in which fluoride was not present in the ration as well as when it was. Moreover it occurred only in the case of pigs 6-9, 24-3, and 39-30, which received the lower concentrations of fluorine. At no time did the pigs show by behavior or appearance any reaction that could be definitely ascribed to the fluorine.

Pig 32-30, receiving 3 per cent of fluorine in its mineral supplement during the test periods, appeared to be in an unusually healthy condition for the first eight weeks of feeding. The rate of gain was quite uniform and amounted to from 8 to 10 pounds per week. This period of time included two calcium fluoride periods, and throughout no feed was refused. Early in the ninth week this pig began to refuse the ration as offered and was never back to its previous consumption. Later a skin disease developed and very unsightly sores appeared, particularly on the feet and legs. The animal was not able to stand properly because of sore feet. It was killed and an examination made by Dr. Robert Graham, of the Division of Animal Pathology and Hygiene. It was observed that the bones of the pig were exceptionally soft, though nothing unusual was noted about the growth of the teeth. Particularly striking was an extreme fatty degeneration of the liver, which may or may not have been an effect of fluorine. In practically every period after the eighth week there were one or two days in which feed was refused. This pig drank an unusually large quantity of water, amounting to 5 to 6 liters per day as compared to 3 to 4 liters for the other pigs.

The refusal of food by pig 6-6, although not limited to fluorine-containing rations, was probably the result of a disturbance of the appetite brought about by the character of the mineral intake, which contained the highest percentage of fluorine tested. In the case of pig 32-30 also there is no reason evident for the serious failure in appetite or for the general toxic effects noted, aside from the fluorine in the mineral (3 per cent). Some significance might be attached to the effects of long confinement under unnatural conditions, but the other three pigs on the lower fluorine levels did not show any such effect.

TABLE 2.—*Daily gain or loss in body weight of pigs, in kilograms, during each balance period, and average gains for all periods and all animals*

Period Nos. and mineral supplement	Pig 6-9	Pig 24-3	Pig 39-30	Pig 32-30	Pig 6-6	All pigs
1, control.....	0.75	1.13	0.75	0.91	0.71	0.85
2, control.....	.68	.36	.78	.58	.32	.54
3, calcium fluoride.....	.55	.75	.55	.42	.71	.60
4, calcium fluoride.....	.71	.58	.65	.81	.42	.63
5, control.....	.49	.58	.65	.58	.49	.56
6, control.....	.62	.87	.57	.55	.49	.62
7, calcium fluoride.....	.55	.62	.52	.62	.45	.55
8, calcium fluoride.....	.68	-.03	.69	.65	.13	.43
9, control.....	.19	.55	.19	-.29	.26	.18
10, control.....	.84	.68	.29	.65	-.16	.46
11, rock phosphate.....	-.26	-.26	.00	-.39	-.03	-.19
12, rock phosphate.....	.26	.52	-.32	.00	.29	.15
13, control.....	.17	.14	.45	.11	-.19	.14
14, control.....	.06	.55	-.13	.00	.10	.12
Average for all periods.....	.45	.50	.40	.37	.28	.41



The rate of gain of all of the pigs is shown by the curves in Figure 1. In Table 2 are recorded the average daily gains in kilograms for each period. The average daily food intakes for the entire experiment are given in Figure 1. Pigs 6-9 and 32-30, though consuming practically the same average amounts of feed daily, gained 0.45 kgm. and 0.37 kgm. daily, respectively. Pig 32-30 received 3 per cent fluorine in its mineral and pig 6-9 received 0.5 per cent. Pigs 24-3 and 39-30 also consumed on an average practically the same amounts of feed. The rate of gain in the case of pig 39-30, receiving 2 per cent fluorine in its mineral, was 0.44 kgm. daily as compared with a gain of 0.50 kgm. for pig 24-3 receiving 1 per cent. On the basis of these two comparisons, where the food intakes are practically the same, the consumption of calcium fluoride and rock phosphate at the higher levels seems to have produced a correspondingly lower rate of gain.

The average rate of gain of pig 6-6 is low compared with that of the other pigs. The food intake of this pig was also less than the intake of the other pigs, which may explain the lower rate of gain aside from any effect of fluorine. However, inspection of the growth curves in

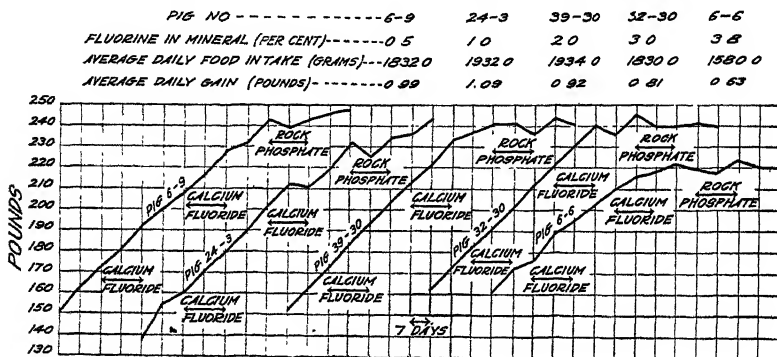


FIGURE 1.—Rate of growth of pigs fed different percentages of fluorine in mineral supplements

Figure 1 shows that there is a progressive decrease in the rate of gain of the individual pigs from pig 24-3, at a 1 per cent fluorine level, to pig 6-6 at a 3.8 per cent level. These results are also indicative of a detrimental effect of fluorine on appetite and on growth, beginning at a level between 1 and 2 per cent of the mineral intake.

Considering Table 2, in which appear the average daily gains in body weight for each pig in each balance period, there are rather extreme differences in the rate of gain shown from one period to another, and such variations may be observed also between periods in which the quality of the ration is exactly the same. It can not be said that any particular variation in weight from one period to another is related to the fluorine intake.

During the first week of the rock phosphate addition, all the pigs either lost in weight or remained stationary. The consumption of food by all of the pigs except pig 24-3 was less during this period, which undoubtedly explains, in part at least, the low rate of gain. None of the pigs returned to their previous level of intake from then on, despite the fact that rock phosphate was not present in the ration of the last two weeks of feeding. These facts suggest a detrimental effect of rock phosphate, even at the lowest level at which it was fed.

There is no evidence that the moisture content of the fecal excretion was affected by the ingestion of calcium fluoride or of rock phosphate, and thus no indication that the calcium fluoride or rock phosphate was either laxative or the reverse.

It seems fair to conclude that, under the conditions of this experiment, the mineral supplements containing 2 or more per cent of fluorine exerted a detrimental effect upon the food consumption and growth of the pigs, and that at levels of 3 per cent or more, distinct deleterious effects resulted. There were indications that rock phosphate exerted a greater detrimental effect than synthetic mixtures of tricalcium phosphate and calcium fluoride containing like percentages of fluorine.

TABLE 3.—Average daily metabolism of calcium by pigs  
PIG 6-9 (BARROW), 0.5 PER CENT FLUORINE IN MINERAL

Period No.	Intake of fluorine	Calcium intake	Calcium in feces	Calcium in urine	Calcium balance	Calcium retained
	Grams	Grams	Grams	Grams	Grams	Per cent
1		5.77	3.94	0.06	1.77	30.6
2		5.83	2.92	.05	2.86	49.0
3	a 0.0611	5.85	2.80	.05	3.04	51.9
4	a .0611	5.88	2.57	.04	3.27	55.6
5		6.08	3.05	.03	3.00	49.3
6		6.08	3.10	.10	2.88	47.3
7	a .0643	6.09	3.30	.08	2.71	44.4
8	a .0643	5.78	2.43	.04	3.31	57.2
9		5.06	2.62	.04	2.40	47.4
10		5.85	2.85	.03	2.96	50.6
11	b .0616	5.20	1.99	.04	3.18	61.1
12	b .0651	5.52	3.14	.04	2.35	42.5
13		5.04	2.26	.04	2.73	54.1
14		4.75	2.50	.05	2.20	46.3

PIG 24-3 (SOW), 1 PER CENT FLUORINE IN MINERAL

1		5.80	3.71	0.07	2.20	38.0
2		5.86	3.39	.05	2.42	41.4
3	a 0.1217	5.92	3.08	.03	2.80	47.4
4	a .1217	5.92	2.94	.04	2.94	49.6
5		6.11	2.85	.00	3.20	52.4
6		6.11	3.45	.04	2.62	42.8
7	a .1283	6.13	3.71	.03	2.39	38.9
8	a .1283	5.71	2.91	.06	2.73	47.8
9		5.21	3.14	.08	2.00	38.3
10		5.73	2.60	.06	3.07	53.5
11	b .1381	5.78	3.38	.06	2.33	40.4
12	b .1381	5.41	2.90	.05	2.45	45.3
13		5.54	2.86	.07	2.60	47.0
14		5.59	2.09	.10	3.40	60.7

PIG 39-30 (BARROW), 2 PER CENT FLUORINE IN MINERAL

1		5.89	3.39	0.07	2.22	37.7
2		5.95	3.45	.04	2.45	41.2
3	a 0.2422	6.02	3.43	.03	2.55	42.4
4	a .2422	6.02	2.82	.05	3.15	52.3
5		6.22	2.87	.05	3.30	53.1
6		6.22	3.26	.03	2.93	47.1
7	a .2557	6.25	3.35	.08	2.82	45.1
8	a .2557	5.92	3.27	.03	2.61	44.1
9		5.71	3.01	.11	2.58	45.2
10		4.94	1.74	.12	3.07	62.2
11	b .2423					
12	b .2625	5.40	2.79	.11	2.49	46.1
13		5.34	2.46	.11	2.78	52.0
14		5.04	2.14	.20	2.70	53.6

a Fluorine present in the form of calcium fluoride.

b Fluorine present in the form of rock phosphate.

TABLE 3.—Average daily metabolism of calcium by pigs—Continued

FIG 32-30 (SOW), 3 PER CENT FLUORINE IN MINERAL

Period No.	Intake of fluorine	Calcium intake	Calcium in feces	Calcium in urine	Calcium balance	Calcium retained
1		5.89	4.10	0.04	1.74	29.6
2		5.95	3.44	.06	2.45	41.2
3	<sup>a</sup> 0.3397	6.03	3.21	.01	2.80	46.5
4	<sup>a</sup> .3597	6.03	2.67	.02	3.34	55.4
5		6.22	2.78	.03	3.40	54.7
6		6.22	3.80	.05	2.36	37.9
7	<sup>a</sup> .3808	6.27	3.20	.06	3.01	47.9
8	<sup>a</sup> .3808	5.94	3.64	.06	2.24	37.6
9						
10		5.62	2.25	.03	3.34	59.5
11	<sup>b</sup> .3836	5.41	3.08	.21	2.11	39.1
12	<sup>b</sup> .3421	4.84	2.67	.10	2.06	42.6
13		5.33	3.00	.13	2.20	41.2
14		3.89	2.11	.08	1.70	43.6

FIG 6-6 (SOW), 3.8 PER CENT FLUORINE IN MINERAL

		5.89	2.27	0.04	3.57	60.6
1						
2						
3	<sup>a</sup> 0.4549	5.87	3.29	.04	2.54	43.2
4	<sup>a</sup> .4185	5.12	2.54	.03	2.55	49.7
5		5.21	2.88	.02	2.31	44.3
6		5.21	2.59	.03	2.59	49.7
7	<sup>a</sup> .4802	6.15	3.37	.03	2.75	44.7
8	<sup>a</sup> .4424	5.38	2.54	.02	2.80	52.1
9		3.98	2.41	.09	1.47	37.1
10		3.89	1.51	.06	2.32	59.7
11	<sup>b</sup> .4566	5.31	2.74	.03	2.55	47.9
12	<sup>b</sup> .5223	5.29	1.86	.04	3.39	63.9
13		4.10	2.13	.04	1.93	47.0
14		4.49	2.14	.05	2.26	50.3

<sup>a</sup> Fluorine present in the form of calcium fluoride.<sup>b</sup> Fluorine present in the form of rock phosphate.

TABLE 4.—Daily calcium balances, in grams, of pigs on different mineral supplements with different fluorine contents, and of control animals

[Averages for duplicate successive periods]

Period Nos. and mineral supplement	Fig 6-9, 0.5 per cent fluorine	Fig 24-3, 1 per cent fluorine	Fig 39- 30, 2 per cent fluorine	Fig 32- 30, 3 per cent fluorine	Fig 6-6, 3.8 per cent fluorine	Average for five pigs
1 and 2, control	2.31	2.31	2.34	2.10	<sup>a</sup> 3.57	2.26
3 and 4, calcium fluoride	3.15	2.87	2.85	3.07	2.54	2.89
5 and 6, control	2.94	2.91	3.12	2.88	2.45	2.80
7 and 8, calcium fluoride	3.01	2.56	2.72	2.62	4.77	2.74
9 and 10, control	2.08	2.53	2.83	<sup>b</sup> 3.34	1.90	2.53
11 and 12, rock phosphate	2.76	2.39	2.49	2.09	2.97	2.54
13 and 14, control	2.46	3.00	2.74	1.95	2.09	2.45
Average of all periods	2.76	2.65	2.74	2.52	2.45	2.61

<sup>a</sup> Period 1 only. This value is not included in averages because of obvious inconsistency with values obtained for other periods. The cause of this high value is thought to be the incorrect estimation of the amounts of the feces and urine excreted during this period.<sup>b</sup> Period 10 only.<sup>c</sup> Period 12 only.

## THE CALCIUM BALANCES

The complete balance sheets for the five pigs appear in Table 3. Table 4 is a summary table of the balance data only.

In planning the experiment it was hoped that a response to the fluorine additions might be obtained within a period of two weeks of fluorine feeding. By determining the calcium balance in control periods before and after the fluorine periods, control data were obtained

for comparison with the balances obtained during each period of calcium fluoride and rock phosphate feeding. It was expected that cumulative effect of fluorine would be manifested in the two successive balance periods constituting the after periods.

The data show that the calcium intake was very constant among the different pigs, as well as among the individual periods for each pig.

The average daily intake for the entire experiment ranges from 5.070 gm. daily for pig 6-6 to 5.773 gm. for pig 24-3. (Table 3.)

In the distribution of the total calcium excreted no differences exist between the feces and urine that may be related to the fluorine content of the ration. In most periods the average daily calcium in the urine for the period is 0.100 gm. or less, ranging from 0.011 to 0.208 gm., both of which values were obtained for pig 32-30. The average daily amount of calcium excreted in the feces varies from 1.51 gm. for pig 6-6 in period 10, to 4.10 gm. for pig 32-30 in period 1. The average daily calcium content of the feces based on all the periods is 2.820 gm., 3.073 gm., 3.938 gm., 3.075 gm., and 2.484 gm. for pigs 6-9, 24-3, 39-30, 32-30, and 6-6, respectively, receiving successively greater amounts of fluorine in their ration. A slightly lower level of calcium intake in the case of pig 6-6 accounts for the smaller amount of calcium in the feces as compared with that in the feces of the other pigs.

It is more or less obvious after examination of the metabolism data, that there was not an immediate response to fluorine additions or withdrawals in the rations of the pigs. Except in the case of pig 6-6, and possibly of pig 32-30, there is no indication that either calcium fluoride or rock phosphate had any effect on the amount of calcium retained. The daily calcium balances for each pig except 6-6 showed a gradual increase up to the end of the fourth week.

In the case of pigs 6-9, 24-3, and 39-30, no significant variations in the balances of the contrasting experimental periods were evident. However, for pig 6-6 the variations in the balances from week to week may be correlated with the calcium fluoride and rock phosphate additions. The balances for the weeks immediately following the calcium fluoride periods, i. e., periods 5 and 9, as well as that for the week immediately following the rock phosphate period, are considerably lower than the balances of the previous periods. Also in the second week after fluorine feeding there is a return to a higher calcium balance.

The balance data for pig 32-30 do not show any variations from period to period which may be correlated with the fluorine intake, either in the calcium fluoride and rock phosphate periods or in the post periods which follow. However, for the last four periods the calcium balances are consistently lower than for any of the other periods. Pig 32-30 refused to eat properly during these periods and made no gain in weight at this time. It is possible that these are the results of a toxic effect of fluorine.

The calcium balances per kilogram of body weight for each balance period, as well as their period averages, do not reveal any more significant differences between fluorine and nonfluorine periods and between pigs receiving different levels of fluorine in their mineral supplements, than do the absolute balances of calcium and their averages, as given in Tables 3 and 4.

The amount of fluorine in the rations of pigs 32-30 and 6-6 was equivalent to about 0.017 to 0.026 per cent of the daily ration and

represented an intake of about 0.0035 to 0.0055 gm. of fluorine daily per kilogram of body weight. The results of feeding 0.017 to 0.026 per cent of fluorine to these pigs may be regarded as rather definite indication that the calcium retention of growing swine may be affected deleteriously by fluorine. Since fluorine was present in the mineral at 2-week intervals only, a more serious effect would probably have followed a régime of continuous fluorine feeding.

#### SUMMARY AND CONCLUSIONS

The deleterious effects noted in the literature as the result of the use of raw rock phosphate as a mineral supplement have been attributed by investigators generally to its fluorine content. An attempt has been made, therefore, to study the effect of fluorine, both as calcium fluoride and as it occurs in raw rock phosphate, on the growth, condition, and calcium metabolism of growing pigs.

It was found that mineral supplements containing 2 per cent or more of fluorine appeared to exert a detrimental effect upon the food consumption and growth of pigs, and that fluorine at levels of 3 per cent or more appeared to cause distinct toxic effects. All mineral supplements were fed in amounts furnishing 5 gm. of calcium daily. There were indications that rock phosphate exerted a more detrimental effect than synthetic mixtures of tricalcium phosphate and calcium fluoride containing like percentages of fluorine.

However, the results of feeding fluorides to pigs, even at the highest levels, that is, about 0.017 to 0.026 per cent of fluorine (0.0035 to 0.0055 gm. per kilogram of body weight), can not be regarded as having demonstrated an effect of fluorine on calcium utilization in intermittent periods of feeding, although there were indications, if nothing more, that these high levels of fluorine depressed calcium metabolism.

The experiment as a whole may be taken to indicate that it is inadvisable to supply the entire calcium requirement of pigs by rock phosphate, but that one-third or less of the requirement fed as rock phosphate may not, in a few months time at least, produce deleterious results. However, before the feeding of rock phosphate even at this level can be recommended it must be shown, not only that it is not toxic, but also that it is as efficient a calcium supplement as bone-meal, limestone, and other products of known value.

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# EFFECT OF THE STRUCTURE AND MOISTURE OF PLANT CONTAINERS ON THE TEMPERATURE OF THEIR SOIL CONTENTS<sup>1</sup>

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## INTRODUCTION

The work presented in this paper is the outgrowth of the observation that the structure of a soil container does influence the soil temperature. The rôle that soil temperature plays in plant growth may not be as apparent as the rôle of air temperature, but any record of air temperature is not a measure of the soil temperature when small containers are used. The experience of this laboratory does not indicate that the choice of a plant container should be influenced by the question of porosity, for as good, if not better, plants have been grown in vitrified containers as in the standard clay pot. Further, the experimental work at this laboratory has not shown that the variable soil temperatures encountered with containers of different structure have any direct effect on plant growth. But soil temperature is of vast importance to the world flora and in particular to the flora of the soil.

## PLAN OF THE INVESTIGATION

In order to secure a fairly even air temperature the apparatus used in maintaining constant soil temperatures was adapted to give a constant air temperature. Long, cylindrical, galvanized-iron pots surrounded by water at a high temperature under thermostatic control made it possible to give each pot the same environment. A wire form held the pot in place suspended over the heated air so that it was equally exposed. The common 3-inch clay flower pot was considered as a standard. This pot is known as a medium-burn and is the type most in demand by growers. The ordinary thick glass tumbler holds the same quantity of soil as does the 3-inch pot and was used in these experiments as an example of a pot made of vitreous material. Three other types of commercial pots were employed in these tests: (1) A pot made of compressed peat; (2) a pot of leather fiber and unknown ingredients; and (3) a pot of heavy paper infiltrated with paraffin.

In preparing for an experiment the pots were submerged in water until the pore spaces had become filled. They were well drained before being filled with soil. Water was added to the soil in excess to insure a water contact between the soil and the pot. The pots were left in the same temperature environment overnight. A thermometer in each pot at the same depth made it possible to determine the temperature of the soil at a locus near the middle of the soil mass. The readings were at half-hour intervals over a period of eight hours.

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The publication of all the data obtained would occupy considerable space. Suffice it to state that many experiments were carried through comparing four standard pots with an equal number of each of the other types. The consistent results obtained showed that the tanks heating the air gave an exceptionally high degree of uniformity.

In Table 1 are given the temperatures of the soil in the various pots for two experiments performed under conditions when there was a considerable difference in the greenhouse temperatures. These two experiments are typical of the many experiments and the greenhouse temperatures are not abnormally high, the maximum being 88° F.<sup>2</sup> in experiment 1, and 101° in experiment 2. The air around each pot was heated by water at 140°, but never reached this point as it was lowered by the air temperature in the greenhouse. The greenhouse air temperatures for the half-hour intervals are also recorded as they indicate certain differences that are apparent in the two experiments, the greenhouse air temperatures ranging from 2° to 15° higher in experiment 2 than in experiment 1.

TABLE 1.—*Temperatures of soil mass in soil containers made of different materials as compared with greenhouse air temperatures*

Time	Temperatures of soil mass in pots of different materials and in the greenhouse in											
	Experiment 1, Mar. 14, 1920						Experiment 2, Mar. 18, 1920					
	Standard pot	Paper pot	Peat pot	Fiber pot	Glass tumbler	Greenhouse	Standard pot	Paper pot	Peat pot	Fiber pot	Glass tumbler	Greenhouse
	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.
8.30 a. m.	64	67	64	65	68	72	61	64	59	62	70	75
9 a. m.	77	82	73	73	82	84	70	75	66	70	81	90
9.30 a. m.	83	91	79	79	93	85	82	86	77	81	95	97
10 a. m.	85	95	82	84	99	86	86	91	82	86	104	97
10.30 a. m.	85	99	84	86	102	85	86	91	82	88	108	97
11 a. m.	85	100	84	88	101	86	88	95	86	91	113	101
11.30 a. m.	86	100	84	90	105	86	91	99	91	95	116	100
12 m.	86	102	85	90	105	86	91	99	90	95	115	93
12.30 p. m.	85	102	84	90	105	88	90	97	88	93	113	91
1 p. m.	85	102	85	91	105	81	86	94	86	91	110	88
1.30 p. m.	85	102	86	91	105	84	86	93	84	91	110	88
2 p. m.	86	102	86	91	105	84	87	94	85	91	111	95
2.30 p. m.	86	102	86	91	105	85	88	95	86	92	111	93
3 p. m.	86	102	86	91	105	84	88	95	86	92	111	91
3.30 p. m.	86	102	86	91	105	84	86	94	84	91	109	86
4 p. m.	86	102	86	91	105	84	85	93	84	91	109	88
4.30 p. m.	86	103	86	91	105	88	86	95	84	91	109	93

The data of experiment 1 are also presented as a graph in Figure 1. It is quite apparent that the porous pots of clay and peat gave the lowest temperatures. The nonporous pots of glass and paraffin-protected paper gave the highest temperatures. The fiber pot is somewhat resistant to the absorption of water and this fact causes its soil temperature to come between those of the vitreous and porous materials.

Particular evidence that the variations of temperature found in the soils contained in pots of different structure is related to water evaporation was determined by an experiment with the peat pots. When

<sup>2</sup> The temperature readings were secured in degrees centigrade but are recorded in degrees Fahrenheit, as this latter scale is the usual measure of air temperature.

peat pots are soaked in water in a nested arrangement (one pot partly surrounded by another) it is practically impossible to moisten them thoroughly. In Table 2 are presented the temperature data showing the relative uniformity obtained in four standard pots and the considerable variation in peat pots that were approximately one-half, two-thirds, and completely moistened. The dry area of the improperly moistened peat pots was the means of determining the approximate area of the nonevaporating surface, which was reflected in the higher soil temperatures found in these pots.

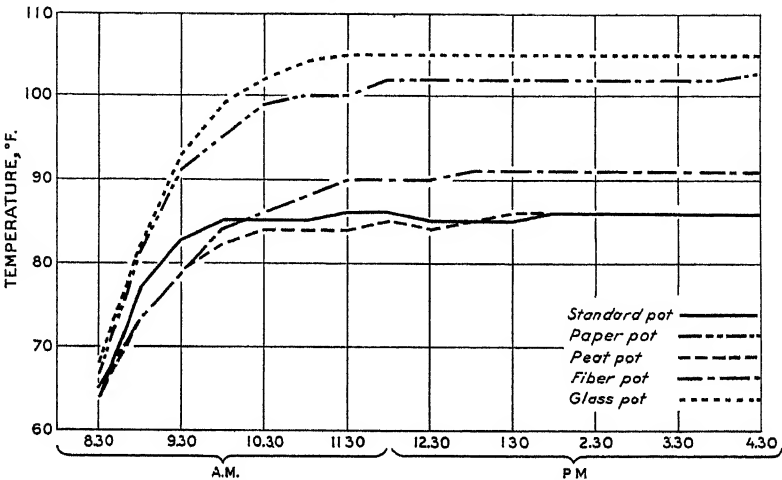


FIGURE 1.—Temperatures of soil in different types of plant containers at half-hour intervals for eight hours

TABLE 2.—Soil temperature readings at half-hour intervals in standard pots uniformly moist and in peat pots not uniformly moist

Time	Temperature in—							
	Standard pots				Peat pots			
	No. 1, 100 per cent moist	No. 2, 100 per cent moist	No. 3, 100 per cent moist	No. 4, 100 per cent moist	No. 1, 50 per cent moist	No. 2, 100 per cent moist	No. 3, 66 per cent moist	No. 4 100 per cent moist
8 30 a. m.	74	75	74	74	76	75	73	72
9 a. m.	79	80	80	79	82	79	79	76
9 30 a. m.	81	81	81	81	84	81	81	78
10 a. m.	82	82	82	82	86	82	82	81
10 30 a. m.	83	84	83	83	88	83	84	82
11 a. m.	84	85	86	84	90	84	86	82
11 30 a. m.	84	86	86	86	90	86	88	84
12 m.	84	86	86	86	91	86	88	84
12 30 p. m.	84	86	86	86	91	88	88	86
1 p. m.	84	86	86	85	91	87	89	86
1 30 p. m.	84	86	86	85	91	87	90	87
2 p. m.	86	86	86	85	91	88	90	87
2 30 p. m.	86	86	86	86	93	88	91	88
3 p. m.	86	86	86	86	92	88	91	88
3 30 p. m.	86	86	86	86	91	88	91	88
4 p. m.	84	85	84	85	91	88	91	88
4 30 p. m.	82	82	82	84	90	86	88	86

Tests were made with clay pots classified according to the degree of firing in their manufacture. These are known as the soft, medium,

hard, and very hard burn. The very hard burned pot has been fired to the point of becoming vitrified and as such had soil temperatures equivalent to those found in the glass tumbler. But there was no variation in the temperatures of the soil in the soft, medium, and hard burned pots. Hence, the assumption seems justified that the cooling effect is more closely related to the area that is moist rather than to any degree of porosity. On the other hand, it is quite reasonable to state that if the element of porosity is lacking the range of temperature is related to the insulating value of the pot structure.

### DISCUSSION

The data here presented show that the soil in a porous flowerpot may have a temperature as much as 20° F. below that found in soil contained in a pot of vitreous material such as glass. The vitreous pots follow, fairly closely, the air temperature of the greenhouse. The principle causing these differences of soil temperature is the latent heat of vaporization. For every gram of water that is changed to vapor about 560 gram-calories of heat are used at the air temperature that surrounded the pots during this study. A considerable proportion of the heat that reaches an evaporating surface is employed in transforming the moisture to vapor and, therefore, the mass has a slower rise in temperature than a similar mass minus an exposed moistened surface. The rise of temperature in containers that are not porous is dependent upon the insulating value of the container.

The cooling effect by evaporation is at a maximum in the porous clay pot. However, when the water of the soil in a clay pot has been reduced to the point where it can not keep the clay pot moist, the temperature of the soil will quickly rise to approximate air temperature.

Through the data presented the author introduces a physical agent altering soil temperatures which may have many applications to research work with soils in small volumes.

### SUMMARY

A method was developed whereby 3-inch plant containers of different materials could be compared with each other under uniform conditions in such a manner that the effect of air temperature on soil temperature was altered by the type of construction of the container.

Pots that were sufficiently porous to produce a moist outside surface maintained a soil temperature lower than the air temperature because of the cooling effect of evaporating water.

Depending on the insulating value of their structure, nonporous containers maintained much higher temperatures than porous containers.

Containers with a part of their surface moist maintained temperatures slightly higher than those with their surfaces completely moistened.

With a uniform air temperature the temperature of soil in small containers depends upon the porosity or insulating value of the material used in the fabrication of the container, and the porous pots are also influenced by the evaporating power of the air.

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## REACTIONS OF THE HONEYBEE TO LIGHT<sup>1</sup>

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### I. EXTENT OF THE SPECTRUM FOR THE HONEYBEE AND THE DISTRIBUTION OF ITS STIMULATIVE EFFICIENCY

#### INTRODUCTION

In considering the reactions of a given animal to light of different wave lengths there are three fundamental questions which perhaps come first to mind: (1) The extent of the spectrum for this animal—that is, the wave lengths of the shortest and longest radiations to which it responds, (2) the relative efficiency of the different wave lengths in eliciting the same response, and (3) the extent to which the animal distinguishes the different wave lengths, or the number of chromas that it distinguishes.

The last of these questions is discussed in Part II of this paper, but the first two may properly be considered now. Before asking these questions in regard to the honeybee in particular, however, it may be well, for comparison, to review briefly our knowledge of the spectra for other animals, so far as investigation has revealed. The results of the more important investigations follow: The spectrum for man (12)<sup>3</sup> is said to extend from about wave length 400  $m\mu$  at the violet end to about 770  $m\mu$  at the red end,<sup>4</sup> the chicken from 500 to 700 (19), the pigeon from 424 to 704 (26), the alligator from 398 to 704 (26), the fish *Phoxinus* from about 340 to about 800 (37), the blowfly larva from about 422 to 625 (30), the water-flea *Daphnia* as low as 228 (3), the cyprid larva of the barnacle *Balanus* from 355 to 690 (36), the clam *Mya arenaria* from about 420 to about 650 (14), the larva of the worm *Arenicola* from 442 to 534 (30), the earthworm from 463 to 514 (30), *Cerianthus* from 460 to about 653 (31), *Volvox* from 434 to 655 (27), and in an investigation of 12 protozoa (30) the spectrum

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<sup>2</sup> Now professor of biology, Western Maryland College, Westminster, Md. The writer is indebted to S. O. Mast, of the Johns Hopkins University, for suggesting the problems here considered and giving general supervision to the work on Parts I and II; to James I. Hambleton, in charge of the division of bee culture, Bureau of Entomology, for making possible the investigations and furnishing laboratory facilities; to A. L. Colton, of the Bureau of Entomology, for helpful suggestions on statistical and mathematical questions; to W. W. Coblenz, of the Bureau of Standards, U. S. Department of Commerce, for determinations of spectral energy; and to all here named for much kindly advice.

<sup>3</sup> Reference is made by number (italics) to Literature Cited, p. 417.

<sup>4</sup> A high limit of 850 has been reported (34, p. 103) and a low limit of 210 (29). The writer can see ultra-violet as low as wave length 365  $m\mu$  when his eyes are fully adapted to darkness.

was found to extend from between 422 and 483 at the violet end to between 504 and 646 at the red end.

A word of caution against taking these and similar data too literally is perhaps advisable here. It must be remembered that the difficulties involved in such studies as those cited are very great. In the first place, it is difficult to compare man with other animals, since with man the subject can speak of his subjective impressions, but with other animals one has to observe some physical response as a proof that the light is having an effect. If the light has an effect which is subminimal to the response which one is observing there is no way of ascertaining that fact. In the second place, owing to the difficulty of controlling accurately the wave length of the light used, it is not easy to determine exactly the limits of the spectrum for an animal. The various light filters often employed, although transmitting much of the incident light, transmit at the same time a wide band of wave lengths, with different proportions of each. On the other hand, the sections of a pure prismatic spectrum such as is sometimes employed, although much more homogeneous in wave length, are often too weak to elicit a response at a particular wave length. In the third place, it is often true that an animal still responds at the shortest or even the longest wave length producible by the apparatus used. In such an instance one obviously can not be sure whether he has reached the animal's limit of vision. And even in case the limit has been reached at a given intensity of illumination, there is no reason to suppose that this is an absolute limit, and would not extend farther if the intensity were increased.

Notwithstanding these and other considerations which make it impossible to state precisely and unconditionally what the extent of the spectrum is for a particular animal, certain conclusions may be drawn. It is apparent that the average human eye under usual conditions is sensitive to radiations ranging in wave length between about 400 and 770  $m\mu$ . The range for man extends on the red end of the spectrum farther than the range for most of the other animals. Several of the experimenters remark on the fact that red light to which they themselves are sensitive does not seem to stimulate the animals in question. However, in birds, and possibly also in fishes, the opposite seems to hold. Honigmann (19) says that for chickens the region around wave length 660  $m\mu$  is four times as stimulative as for man, and the fish *Phoxinus laevis* has been credited with being sensitive to radiations of wave lengths nearly or quite as long as 800  $m\mu$  (37). On the other hand, it is fairly common to find animals sensitive to the shorter wave lengths in the deep blue and violet, e. g., at 425  $m\mu$ . Some are said to exceed man in this respect, the fish just mentioned being sensitive to radiations at 340  $m\mu$  and *Daphnia* even to those at 228  $m\mu$ .

But what of the honeybee in particular? There has been no direct research on this problem with honeybees, but there is considerable evidence to be derived from the so-called training experiments of Von Frisch (7) and Kühn (22). These men first trained the bees to associate a given color with food, and then observed whether or not the bees confused that color with other colors or with various shades of gray. Von Frisch, for example, found that after training bees to come for food placed exclusively on red paper, and then after shifting the position of the red in relation to that of various gray papers presented at

the same time, the bees confused dark gray and black with red, although they did not confuse yellow, or blue, or violet, with any gray. This result indicates that the paper which appears red to us appears very dark, almost black to bees; in other words, that their spectrum does not extend as far into the red as our own.

Kühn (22), using spectral colors instead of colored papers, got results essentially similar to those of Von Frisch in regard to red. More precisely, he maintains that the bee does not respond to wave lengths longer than  $650\text{ m}\mu$ . In addition, however, he found that his bees were stimulated by regions of the ultra-violet as low as that of wave length  $313\text{ m}\mu$ , ordinarily invisible to man. And not only were they stimulated, but after being trained to associate the wave lengths  $313\text{ m}\mu$  and  $365\text{ m}\mu$  with the presence of food, they did not confuse these two regions with any other region of the spectrum which he tried—a result which indicates that they see this ultra-violet light as a color distinct from all colors visible to us, and not merely as a fluorescence, and that, consequently, the spectrum visible to them extends at least as far into the ultra-violet as the wave length  $313\text{ m}\mu$ .

In regard to the honeybee, nothing at all has been known as to what region of the spectrum is most efficient in stimulating it. If it is true that the spectrum visible to the bee extends from wave length  $313\text{ m}\mu$  in the ultra-violet up to  $650\text{ m}\mu$  in the orange-red, we should expect to find the maximum point, judging from the usual results, somewhere more than halfway toward the ultra-violet end of the spectrum, probably in the blue. Or perhaps there are two maxima, one in the usual green or greenish-yellow region and another in the violet or ultra-violet. To determine the relative stimulative efficiency of the various parts of the spectrum to which the bee reacts, and to ascertain more carefully its limits, thus become especially alluring problems. The experiments to be described were designed to throw some light on them.

In regard to the second question mentioned at the beginning of this introduction—namely, the relative stimulative efficiency of different wave lengths—comparatively little is known except with respect to the human eye. For man a summary of the various investigations (12) places the point of maximum efficiency between the wave lengths  $550$  and  $560\text{ m}\mu$ . This standard curve is reproduced in Figure 4 (dotted curve). For other animals the most reliable investigations give the following results: For the chicken (19, p. 68) the maximum efficiency is between  $560$  and  $580\text{ m}\mu$ , for the pigeon (26) it is at  $564$ , for the alligator (26) it is at  $544$ , for the fish *Phoxinus* (37) it is probably at  $592$ ,<sup>5</sup> for the blowfly larva (30) at  $504$ , for the cyprinid larva of *Balanus* (36) at about  $540$ , for *Mya arenaria* (14) at about  $500$ , for the *Arenicola* larva and the earthworm (30) at  $483$ , for *Cerianthus* (31) at about  $532$ , for *Volvox* (27) at about  $494$ , and for various protozoa (30) it ranges between  $534$  and  $473$ , the location depending on the animal. Taken as a whole, the region of the spectrum most efficient in stimulating animals, so far as these data show, is somewhere between the blue-green and the yellow region; in other words, somewhere between the wave lengths  $473$  and  $580\text{ m}\mu$ , according to the particular animal. Only in birds and possibly in fishes does the maximum lie farther toward the red end of the spectrum than in man; in all others it lies

<sup>5</sup> This is the region which the fish most readily learns to associate with food; it may not be the region of actual maximum efficiency.

farther toward the violet end. Where complete curves of the distribution of stimulative efficiency in the spectrum have been worked out they are similar to the curve for man presented in Figure 4. In the curve for any given animal the maximum is nearly always not exactly in the center of the curve, but lies slightly skewed toward the violet end.

#### METHODS AND RESULTS

One of the most successful methods of finding the relative stimulative efficiency of various wave lengths which are unequal in energy is the one used by Mast in 1917 (30). The procedure is, briefly, as follows: The animals are exposed simultaneously to a spectral color and to white light of a measurably variable intensity. This intensity is then varied until the animals react equally to both beams. Then another spectral color is chosen and again the white is equated to it, and so on for as many as may be desired, or as is possible with the apparatus at hand. The relative intensities of the white then represent the relative stimulation caused by the various spectral colors. It should be emphasized that the white is adjusted until its effect on the animal is *equal* to that of each spectral color used; this is a point that will be referred to later. Having found the relative *effect* of each spectral color, the relative *efficiency* of each can then be calculated by simply dividing the relative effect by the relative energy transmitted at that wave length.

This method, in essentials, was used in the experiments here described, although several modifications had to be made, which will be considered at the proper place.

At the bee culture laboratory there was available a spectroscope of the constant-deviation type, with which to obtain light of the wave lengths desired. A 400-watt gas-filled projection lamp having a tungsten filament furnished the light for the spectrum. The width of the collimator slit of the spectroscope was made 1.8 mm. and that of the telescope slit 1.05 mm. The graduated head of the instrument, directly denoting the wave length of the light used, read from 430  $m\mu$  to 700  $m\mu$ . The readings for these experiments were chosen at intervals of 20  $m\mu$  from 430  $m\mu$ , giving 13 steps up to and including 690  $m\mu$ , or, in all, 14 wave lengths. On subsequent calibration of the instrument with a helium tube, the indicated wave length 430  $m\mu$  was found to be really 431  $m\mu$ , and 690  $m\mu$  to be really 697.5  $m\mu$ , with intermediate points proportionately in error; the corrected values are therefore substituted for those indicated by the instrument. It is recognized, of course, that a slit 1.05  $\mu$ m. in width will not give monochromatic light. The length of spectrum able to pass a telescope slit of given width is, in a prismatic spectrum, greater for the longer waves than for the shorter waves, owing to the greater refraction of the latter. Thus, in this apparatus the light said to have a wave length of 431  $m\mu$  was in reality the aggregate of a band extending approximately from 429  $m\mu$  to 433  $m\mu$ , and similarly that called 690  $m\mu$  was a band extending approximately from 675  $m\mu$  to 705  $m\mu$ , those between varying proportionately.

The first problem was the arrangement of an apparatus in which the bees would react primarily to light and not to other stimuli. For example, one of the most disturbing factors in such work as this is the tendency of bees in close confinement simply to race continuously around the sides of the container, apparently in a frantic effort to escape, and to disregard the light almost entirely. It was

found that to remedy this difficulty the container must be made so large that even with its great activity the bee will seldom strike the sides, but will be able to roam in comparative freedom over a large space. Every effort to confine the bee to narrow paths or make it go through small openings resulted in almost complete substitution of contact reactions for light reactions. After a number of tests the need was satisfactorily met. A shallow, rectangular box, 65 by 25 by 4 cm., was made and covered with a wire screen. Through a glass-covered opening in the middle of one end of this box a narrow beam of light from an ordinary electric bulb could be projected, and through two openings in the opposite end, each closed with a diffusive screen of ground glass, the light from the spectroscope and white light from a comparison lamp could be projected simultaneously, the two screens being of the same shape and size. Each screen was at the end of a tunnel, the two tunnels extending across the

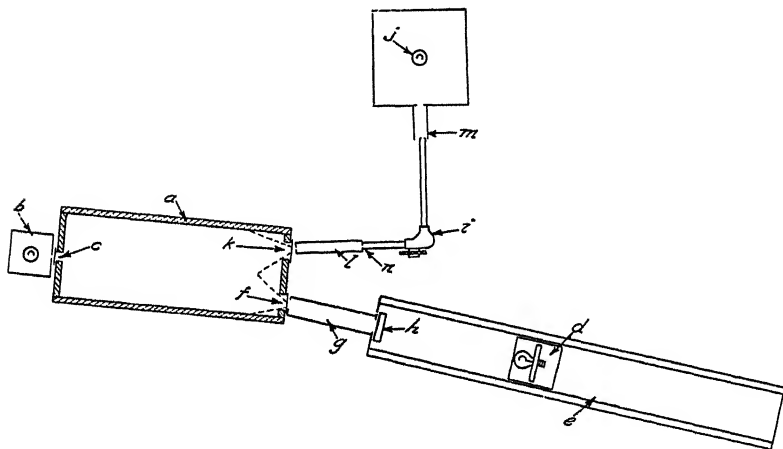


FIGURE 1.—Arrangement of apparatus used to ascertain the relative stimulative effect of spectral rays upon bees; *a*, box in which the bees were inclosed for experimentation; *b*, box containing electric bulb to illuminate ground glass at aperture *c*; *d*, comparison lamp, inclosed in box *e*, and illuminating ground glass at aperture *f*, through tube *g*; *h*, cell containing solution of cupric sulphate; *i*, spectroscope, illuminated by electric lamp *j*, and directing beam of colored light on ground glass at aperture *k* through tube *l*; *m*, collimator slit; *n*, telescope slit

width of the box. The apparatus was, of course, set up in the dark room.

The diagram, Figure 1, is inserted to convey a better idea of the arrangement of the apparatus. The box *a* is 65 by 25 by 4 cm. in size, and covered with a wire screen; in this the bees were confined; *b*, a box, 15 by 15 by 15 cm., in which was placed a 15-watt electric bulb, to illuminate a piece of ground glass which covers the aperture *c* connecting *a* and *b*; at the end of *a*, opposite this aperture, are two passages, with flaring sides, leading to the two apertures *f* and *k*, each covered with ground glass. The ground glass at *f* was illuminated by the 100-watt "comparison lamp" *d*, which was inclosed in a box, *e*, 117 cm. long and provided with guides for conveniently adjusting the distance of the lamp from the ground glass. The only light escaping from this box was emitted through an opening in one end of it, 26 cm. from the ground glass, this opening and the aperture *f* being connected by a tube *g* to prevent



diffusion of the light. At the end of this box, covering the opening, was a glass vessel, *h*, containing a dilute solution of cupric sulphate, to eliminate infra-red light. The glass of this vessel and of the diffusion screens also eliminated ultra-violet. The aperture *k* was connected by a tube *l* with the slit through which the light of the wave length chosen was emitted from the spectroscope.

As has been stated, the spectroscope *i* is of the constant-deviation type, with a graduated head for controlling the wave length of light to be emitted by the slit *n* and thrown on the ground glass at the aperture *k*. The collimator slit *m* was illuminated by a 400-watt electric lamp, *j*, inclosed to prevent the escape of light and placed at a suitable distance.

As a beginning of the routine of experimentation, 10 bees were caught at a feeder or on flowers<sup>6</sup> and were put into the box. A feeder, consisting of a bottle of sugar water, its mouth covered with cloth, was then inverted on the wire screen and the light of the dark room turned off. The bees soon collected on the cloth and began to feed. They were left in darkness for from three to five minutes; in this time they became satiated with sugar water, and quieter and less excitable. They also became somewhat adapted to the darkness. The small lamp (in box *b*, fig. 1) illuminating the opening at the center of one end of the box was then turned on and left until the bees congregated at this end, then turned off, and simultaneously the spectroscope lamp and the comparison lamp were turned on, each producing an illuminated area of the same size on the ground-glass screen used with it at the opposite end of the box. Most of the bees then went toward these areas, part entering one of the two flaring tunnels (fig. 1, *f* and *k*) and part the other, sometimes a few entering neither, but wandering aimlessly, without direction. After the bees entering each tunnel had been counted, the lamp at the opposite end was again turned on and the opening there illuminated until the bees congregated at that end (fig. 1, *c*), then the whole process was repeated. The 10 bees were thus induced to react over and over again until they began to appear sluggish and somewhat indifferent to the light. Ordinarily about 150 positive reactions were obtained from 10 bees. As soon as the bees became unfit for further use they were removed from the box, taken into the open, and released. Then 10 more field bees were captured and put into the box, and 150 positive reactions again obtained, and this procedure was continued as long as desired.

In the earlier experiments an attempt was made so to adjust, by changing the distance of the lamp, the intensity of the beam of white light that the response induced by it equaled that induced by the beam from the spectroscope, but this was found to be almost an endless task. After spending a whole day in counting reactions with the comparison lamp set at a given distance, it was usually found that the response induced by it was not equal to that induced by the given spectral color; so, after slightly increasing or decreasing the distance, as the results demanded, the experiment had to be continued for another day to see if the two beams were now equal; if they were not, the distance had again to be changed and another day spent, and so

<sup>6</sup> Field bees must be used. Young bees that have not yet become field bees are practically indifferent to light.

on. One was never quite sure when the true value had been obtained—never quite sure when to stop.

To avoid this difficulty the method was modified. Instead of varying the intensity of the white light on the ground glass in an effort to make its effect equal to that of the spectral light, the comparison lamp was left at a constant distance and its effect on the reactions of the bees was compared with the corresponding effect of each spectral color. The graduated head of the spectroscope was turned to indicate a given wave length—e. g.,  $698\text{ m}\mu$ —and, after the bees had been induced to assemble at the opposite end of the box, the beam from the comparison lamp and light of the wave length  $698\text{ m}\mu$  were thrown on the two screens simultaneously. The bees entering each of the tunnels were counted; then the bees were brought back to the starting point again and the process repeated until a total of 25 bees had entered the two tunnels. Then the graduated head was set for another wave length and the procedure repeated, and so on until each of the whole series of 14 different spectral colors had been compared with the constant white. Then the series was repeated, using the 14 spectral colors again, and so on for 20 series—i. e., until the total number of entrances (into both tunnels) observed in the comparison of each spectral color with the white amounted to 500.

For each wave length, in each series, a total of 25 bees, as has been said, entered the two tunnels. Only those entering the tunnel leading to the ground glass illuminated by the spectral color were recorded. To find the relative stimulative effect of light of a given wave length as compared with the stimulative effect of the white light used for comparison, the total number of bees recorded for a given wave length was divided by the corresponding total of those attracted to the white light; in other words, by the difference between 500 and the total recorded. For instance, in the 20 series, a total of 70 bees were attracted to the ground glass by light of wave length  $431\text{ m}\mu$ . By dividing 70 by 430 (i. e.,  $500 - 70$ ) the ratio 0.16 is derived as the relative stimulative effect of light of this wave length as compared with the white light taken as a standard. The results obtained are presented in Table 1. They indicate that, beginning with the shortest wave length and proceeding to the longest, the stimulative effect gradually increases to a maximum at  $553\text{ m}\mu$  and then decreases.

But a serious question arises here: Do the ratios obtained in this way represent the true relative stimulative effect of the respective wave lengths? For example, if the ratio for one wave length is twice that for another, is the stimulative effect of the former really twice that of the latter? At first thought the answer appears to be in the affirmative, but further consideration shows that these values may *not* truly represent the relative stimulative effect of the various wave lengths. For, according to the method used by Mast (30), already described, the true relative stimulative effects of diverse wave lengths are the relative intensities of white required to produce responses equal in magnitude to those produced by the several wave lengths used.

TABLE 1.—Record of the number of bees attracted by light of selected wave lengths, and ratios of the totals for each wave length to the corresponding totals for the white light which was taken as the standard of comparison

Series	Number of bees attracted to light of wave length specified (in millimicrons)													
	431	451	471	492	512	532	553	574	595	615	636	657	677	698
1.....	3	6	6	8	11	10	11	15	11	7	4	4	3	1
2.....	2	7	6	9	10	8	12	8	6	9	3	4	6	1
3.....	6	4	7	6	14	10	11	8	9	6	8	4	3	1
4.....	7	1	6	9	10	13	13	14	8	6	4	5	2	5
5.....	0	5	9	8	8	12	13	14	9	10	7	4	6	3
6.....	2	4	5	10	8	15	11	14	14	9	7	2	0	2
7.....	5	4	8	7	12	18	16	16	9	10	6	4	4	0
8.....	4	4	9	8	10	15	14	13	15	6	5	5	2	0
9.....	6	9	8	10	8	10	14	11	14	9	8	6	3	2
10.....	5	6	9	10	15	13	14	13	12	13	7	3	2	0
11.....	4	5	10	11	13	10	12	12	8	9	6	4	3	3
12.....	3	4	7	12	7	10	13	13	11	8	7	6	5	0
13.....	6	7	8	11	10	10	14	13	9	9	7	3	2	1
14.....	5	3	9	8	11	11	12	8	14	8	5	5	4	2
15.....	1	3	6	8	10	9	13	9	11	9	7	4	5	6
16.....	0	6	7	10	13	13	12	9	8	5	4	5	3	2
17.....	2	7	4	11	14	13	11	11	11	10	4	5	3	4
18.....	4	5	5	9	9	6	10	10	13	13	3	3	2	2
19.....	4	6	7	11	8	12	13	13	10	6	5	1	4	1
20.....	1	3	6	6	10	10	11	14	11	9	4	3	3	3
Total.....	70	99	142	182	211	228	250	238	213	171	111	80	65	39
Total attracted to white light.....	430	401	358	318	289	272	250	262	287	320	389	420	435	461
Ratio.....	0.16	0.25	0.40	0.57	0.73	0.84	1.00	0.91	0.74	0.52	0.29	0.19	0.15	0.085

Consequently, the question that now arises is this: If a given wave length induces, for example, 25 per cent as great a response as a given white, how much will the white have to be reduced to bring about a response equal to that of the given wave length? Or, we may state the question in another way and ask, if a white beam be substituted for the spectral color, how intense will this white beam be, relative to the constant white, when it induces 25 per cent as many positive responses as the latter? In other words, what is the relation between the relative intensity of two beams of white light and the relative magnitude of the responses induced by them?

This relation was found without much difficulty. The apparatus used for this purpose was the same as that represented in Figure 1, except that for the spectroscope unit there was substituted another unit just like the comparison lamp and its container, so as to produce two beams of white light against the diffusion screens. The beam coming from the comparison lamp was left constant, while that coming from the new unit was varied in intensity through eight steps, ranging from approximately three times the intensity of the comparison lamp down to zero, either by substituting a more powerful lamp, by changing the distance of the lamp, or by interposing a neutral tint filter of carefully measured transmission, or, finally, by turning off the lamp entirely.

The experiment was conducted practically as has been described; that is, the bees, after being placed in the box and fed, were caused to assemble at the opening illuminated by the small pilot lamp (fig. 1, c), then the two lamps illuminating the pieces of ground glass at the opposite end of the box were turned on and the pilot lamp was turned off, after which the bees entering each of the two tunnels leading to the ground glasses were counted. The bees were now

again brought together at the starting point, by turning on the pilot lamp and extinguishing the other two; then the pilot lamp was turned off and the other lamps on, after which the bees entering each tunnel were again counted. The cycle was repeated again and again, new bees being used from time to time, as already described, until a total of from 500 to 2,000 bees had entered the two tunnels for each of the eight values of the variable illumination used.

Since the purpose of this experiment was to find the relation between the relative intensity of the variable beam and the relative number of bees going toward the spot illuminated by it, the results are tabulated as two ratios: (1) The ratio of the intensity of the variable to that of the constant beam, and (2) the resulting ratio of the number of bees attracted by the variable beam to the number attracted by the constant beam. The intensity ratios, for the eight different intensities of the variable beam used, are 2.98, 1.00, 0.50, 0.25, 0.11, 0.05, 0.01 and 0; the ratios of the effects on the bees corresponding to these are 1.67, 1.01, 0.85, 0.66, 0.50, 0.40, 0.20, and 0.09. These ratios and a summary of the observations on which they are based are presented in Table 2.

TABLE 2.—*The ratios of intensities of a variable beam of light to the intensity of a constant beam, tabulated for comparison with the corresponding ratios of the numbers of bees attracted to each light*

Ratio of intensity of variable beam to that of constant beam	Total number of bees observed	Number of bees attracted by variable beam	Number of bees attracted by constant beam	Ratio of number of bees attracted by variable beam to number attracted by constant beam
2.98	1,000	625	375	1.67
1.00	1,000	502	498	1.01
.50	1,000	459	541	.85
.25	1,500	597	903	.66
.11	1,000	333	667	.50
.05	2,000	576	1,424	.40
.01	500	85	415	.20
-----	500	40	460	.09

The data presented in Table 2 show clearly the following facts: When the two intensities of the pair are equal (column 1, ratio 1.00) the stimulative effect produced by each is practically the same (last column, ratio 1.01), as one would expect. But when the intensity of the variable beam is changed, the effect does not change proportionally; for example, when the intensity of the variable beam is decreased so that its ratio to that of the constant beam is 0.11 (first column), the effect produced decreases only to a ratio of 0.50 (last column). For unequal illumination the departure from direct proportionality is evident throughout. It will be observed that even when the intensity of the variable beam is reduced to zero (lamp turned off entirely), some bees enter this unlighted tunnel. This behavior is due partly to the activities of indifferent bees—bees which, after being confined for a little while, seem to lose their photopositivity and simply roam aimlessly about, sometimes entering the unlighted tunnel; it is due partly also to the flaring shape of the

tunnels, which sometimes catch bees coming in from one side of the box toward the tunnel on the opposite side.

The relation between intensity of illumination with white light and magnitude of response on the part of the bees is brought out more clearly when the relative magnitude of response is plotted as a function of the relative intensity of the variable illumination which produced it. The resulting curve (fig. 2) is not a straight line, but is suggestive of a parabola; however, if the logarithms of the related variables are plotted, an approximately straight line results which correspondingly represents the equation

$$\log R = m \log I + b,$$

where  $I$  is the ratio of the intensity of the variable white light to that of the constant white light,  $R$  the ratio of the number of bees attracted by the variable light to the corresponding number attracted

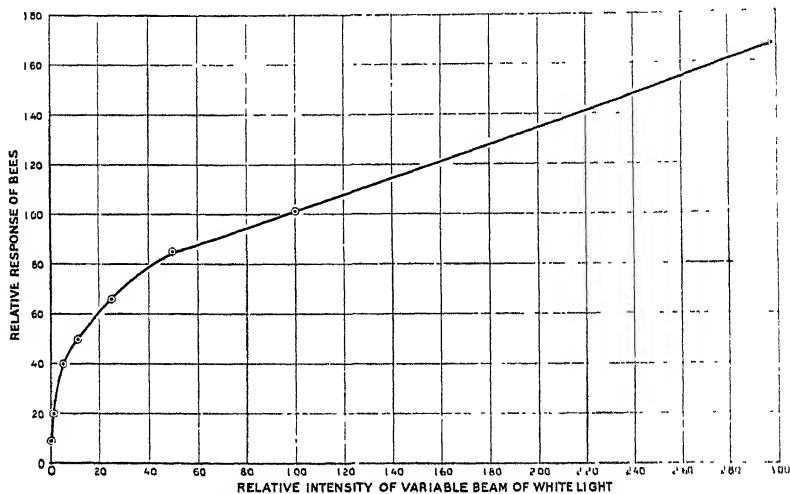


FIGURE 2.—Correlation of the relative intensity of a variable beam of white light and the relative number of bees apparently responding to it

by the constant light,  $m$  the tangent to the angle of inclination, and  $b$  the  $y$  intercept (a constant).

It may be pointed out, in passing, that neither does a straight line result when the relative response is plotted as a function of the logarithm of the relative intensity instead of the relative intensity itself; the resulting curve, shown in the lower part of Figure 3, makes it evident that the relation considered here is not in agreement with the Weber-Fechner law, according to which the magnitude of response increases as the logarithm of the stimulus. Only when the logarithms of both the relative intensity and the relative response are plotted does an approximately straight line result. This approximate proportionality between the logarithm of the stimulus of white light and that of the corresponding response on the part of the bees, a kind of by-product of the present research, seems worth a passing allusion for its mathematical interest. The line would have been perfectly, instead of approximately, straight if the curve in Figure 2 had been

exactly a parabola, and would have been inclined at a different angle. For the greater convenience and simplicity of plotting logarithms which are strictly positive quantities, and inasmuch as only the *relative* values of the intensities of light and of the observed reactions are concerned, the ratios plotted in Figure 2 have throughout been multiplied by 100, and the logarithms of the resulting products are those plotted in Figure 3.

It now becomes possible by the use of the curve shown in Figure 2 to find by graphic interpolation how intense a beam of white light must be, relative to the intensity of the beam from the comparison lamp (fig. 1), in order to produce the same effect as that produced by any one of the spectral colors. The ratio of response induced by the

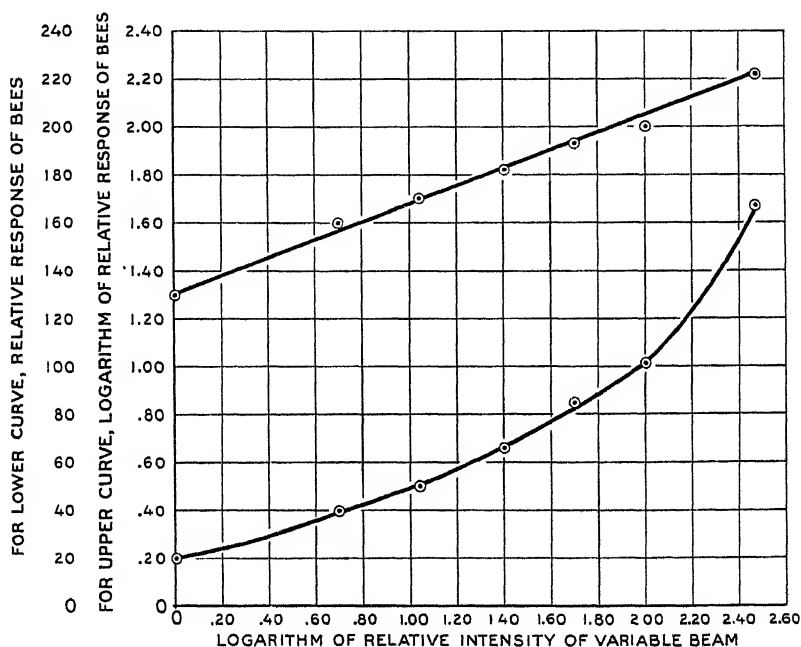


FIGURE 3.—Lower curve, relative response of bees as a function of logarithm of relative intensity of variable beam of white light; upper curve, approximately straight line found by plotting logarithm of relative response of bees as a function of logarithm of relative intensity

spectral region, for example, of wave length  $451 \mu$  is found from Table 1 to be 0.25. Locating this value on the y axis, the corresponding relative intensity of white light is found by the curve to be approximately 0.022, as read off on the x axis. Similar values for the light of each of the 14 spectral bands used in this research were found by this method from a carefully drawn curve like the one in Figure 2 but made on a suitably large scale. In Table 3 are shown, in the first column, the wave lengths of the 14 spectral bands; in the second, the relative stimulative effect of their several colors, taken from the last line of Table 1; and in the third, their relative stimulative values in terms of the constant white illumination, as found by the method of graphic interpolation just described.

TABLE 3.—Steps in the derivation of the relative stimulative efficiency of selected spectral colors in producing reactions of bees

Wave length	Relative stimulative effect <sup>a</sup>	Relative stimulative value <sup>b</sup>	Relative energy at slit of spectroscope <sup>c</sup>	Relative stimulative efficiency	Relative stimulative efficiency, with maximum at 100
$M\mu$				<i>Per cent</i>	<i>Per cent</i>
431	0.16	0.005	0.18	2.78	10.11
451	.25	.022	4	5.50	20.00
471	.40	.060	.8	7.50	27.27
492	.57	.163	1.2	13.58	49.38
512	.73	.330	1.8	18.33	66.65
532	.84	.525	2.5	21.00	76.36
553	1.00	.990	3.6	27.50	100.00
574	.91	.700	4.7	14.89	54.15
595	.74	.345	6.4	5.39	19.60
615	.62	.125	8.0	1.56	5.67
636	.29	.030	10.1	.30	1.09
657	.19	.009	12.8	.07	.25
677	.15	.004	16.0	.025	.09
698	.085	0	19.5	0	0

<sup>a</sup> From last line of Table 1.<sup>b</sup> Found by interpolation on the curve of relative response as a function of relative intensity of illumination. (Fig. 2.)<sup>c</sup> Determined by W. W. Coblenz.

The values last named are those which are necessary for ascertaining the relative *efficiency* of each of these spectral colors, these relative efficiencies being the ultimate object of the research. They are found by dividing the relative stimulative values shown in column 3 by the relative expenditure of energy in the corresponding spectral color; the several quotients represent the relative efficiencies sought. The relative expenditure of energy in each of the 14 wave lengths used was determined for the writer by W. W. Coblenz, of the United States Bureau of Standards, with a delicate thermopile and galvanometer, and the results are shown in the fourth column of the table. In the fifth column are shown the relative stimulative efficiencies sought, the several quotients having been multiplied by 100 in order to be shown as percentages, and in the sixth column the same relative efficiencies after the maximum efficiency has been given an arbitrary value of 100 and the others a proportionate value; in both these columns the values are given as percentages. It will be seen that the efficiency is very low in the red, not attaining a value of 1 per cent of the maximum until the wave length 636  $m\mu$  is reached; from there it rises rather rapidly to a maximum at 553  $m\mu$ ; from this point it decreases more gradually until at 431  $m\mu$ , the shortest wave length obtainable with the spectroscope used, the efficiency is approximately 10 per cent of the maximum.

The distribution of relative stimulative efficiency in the spectrum is made much more evident if these values are plotted as functions of their respective wave lengths and a curve drawn connecting the points. (Figure 4, solid line.)

A comparison of the curve for the bee with that for man, presented in Figure 4, shows that their respective maxima are at about the same wave length, but that the efficiency of the longer waves is greater for man than for bees, and the efficiency of the shorter waves less. The curve for man, as shown in the illustration, has been drawn from

data compiled by Gibson and Tyndall (12, p. 173), of the United States Bureau of Standards.

#### CONCLUSIONS

The upper limit of the spectrum for the honeybee extends to at least wave length 677  $m\mu$ . Kühn (22, p. 799) found evidence of its extent to only about 650  $m\mu$ . The difference between these assigned limits is probably due to the difference in intensity of the spectral light used in the two sets of experiments. The lower limit was not ascertained in the research here reported, but according to Kühn it extends to at least 313  $m\mu$ .

The point of maximal stimulative efficiency is at about 553  $m\mu$ , which corresponds rather closely with that for man. From this point the efficiency decreases more rapidly for bees than for man toward the

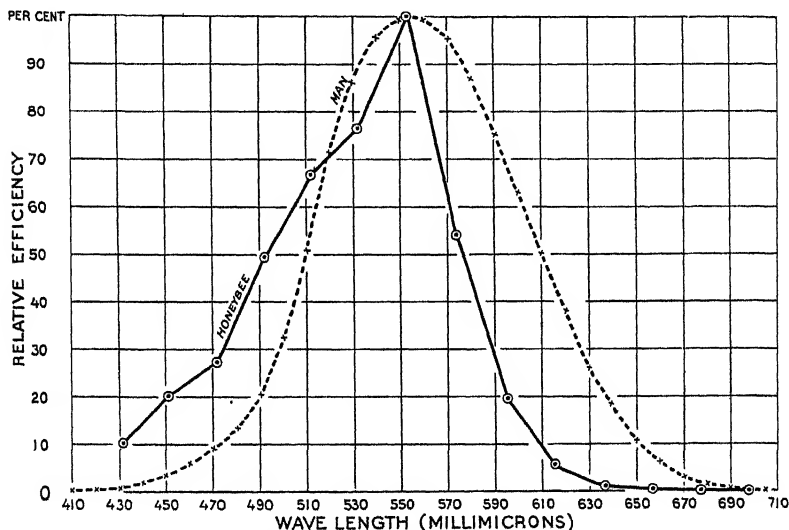


FIGURE 4.—The distribution of relative stimulative efficiency in the spectrum for the honeybee compared with similar distribution in that for man

longer wave lengths, but more slowly than for man toward the shorter wave lengths, being at 431  $m\mu$  still fully 10 per cent of the maximum.

Untrained bees, when allowed to walk toward two sources of light of the same quality, placed near together at the end of a rectangular box, go to the brighter more often than to the fainter, but not in direct proportion to the relative brightness of the two. The curve representing the relation between relative brightness (energy) and relative magnitude of response is a polynomial one, of such form that the curve showing the relation between the logarithms of the two related variables is an approximately straight line.

## II. CHROMA VISION OF THE HONEYBEE

#### DISCUSSION OF TERMS

In almost any paper involving the subject of light and color it is necessary to state clearly the sense in which various terms are to be used. This need arises from the lack of agreement among present-



day workers in the field of optics, human psychology, and general physiology as to the meaning of these terms.

A fundamental distinction is to be made between the terms "light" and "color;" the former is the name of a form of energy, entirely objective, while the latter is the name of a sensation, entirely subjective.

Light has three attributes—brightness, wave length, and purity. Brightness refers to the intensity of illumination as measured in some physical unit such as meter candles or ergs per square centimeter per second. In this paper the term is used synonymously with intensity.

Wave length refers to the dominant wave length of the portion of the spectrum under consideration; in practice it is usually impossible to obtain light of a single wave length. The unit of measurement here used is the millimicron.

Purity refers to the proportion of white light which is, in a given instance, mixed with the spectral light in question; the less the admixture of white light the greater the purity.

Likewise, color has three attributes—brilliance, hue, and saturation. We may, however, combine the last two, as will be done in this paper, and say that color has the two attributes, brilliance and chroma.

Brilliance is that attribute of a color which enables it to be classified as equivalent to some member of a series of grays, ranging between black and white. It is the quantitative aspect of color.

Chroma involves the hue—that is, the redness, yellowness, greenness, blueness, etc., of a color—together with the distinctness or vividness of the hue. It is the qualitative aspect of color.

Color, then, is merely a subjective phenomenon; a sensation that arises from the activity of the photoreceptors and their attached nervous mechanism. It includes the sensations not only of chroma (red, yellow, green, blue, and all combinations of these, for the human eye) but also of gray (the whole series from black to white, including the latter). The meaning of these terms becomes more evident if they are viewed in tabular form. The following arrangement of terms here discussed is a modification of one presented by the Progress committee on spectrophotometry for 1922-3 of the Optical Society of America (33):

Light (objective) evokes the sensation of color (subjective).

Brightness evokes the sensation of brilliance.

Wave length } evoke the sensation of chroma { hue  
Purity } saturation.

If these definitions are adhered to, it will be seen that the terms "color blindness" and "color vision" lose entirely their usual meanings, the former coming to mean entire blindness, and the latter simply light vision; hence in this paper the terms "chroma blindness" and "chroma vision" will be used to express what is ordinarily meant by color blindness and color vision, respectively.

Chroma vision<sup>7</sup> may be defined, then, as the ability of an animal to distinguish between chromas of the same brilliance.

It may be argued that the terms "color," "brilliance," and "chroma," which describe purely human sensations, should not be used when referring to other animals than man, because we do not know what

<sup>7</sup> The use of the term "chroma" to avoid the ambiguities in the meaning of color, has been advocated by Ladd-Franklin (25) and Troland (35).

kind of photic sensations they have, if, indeed, they have any. That is true, of course, but as a matter of fact, even among human beings, no individual knows exactly how light appears to another individual, and yet we find the terms convenient in talking about these sensations. Likewise, in this paper, the terms are used only for convenience, and it is to be understood that there is no intention of attributing to insects the light sensations of human beings. When, for example, the writer speaks of bees being able to distinguish a blue from all shades of gray, he means colors that are called blue and gray by human beings, and no assumption is made as to what sensations they give to bees.

#### REVIEW OF LITERATURE

The method which has always been used in investigating chroma vision of bees is the so-called training method, although several other methods have been used for other animals.<sup>8</sup> The method consists essentially of two procedures: (1) The training of the bee to come for food to a place where food is associated with a given chroma, and (2) the testing of the bee, by some change in the layout of the experiment, to see if it can select from other colors (i. e., chromas or grays) of equal brilliance the particular chroma used in training.

The training of bees to come to almost any desired experimentally arranged set-up is usually not difficult, neither is the evidence that bees select a given chroma from other colors difficult to obtain. But it is very difficult to make sure that at least one of the test colors is of the same brilliance to the bee as the training chroma.

Since chroma vision is, by definition, the ability to distinguish between chromas of equal brilliance, the question persistently arises in a critical review of previous experiments, were the colors used in the tests of the same brilliance to the bees as were the chromas used in the training?

Lubbock (1) and his contemporaries clearly established the fact that bees and certain other insects can under certain conditions distinguish one chroma from another, but they did not ascertain whether this distinction takes place on the basis of chroma alone or on the basis of relative brilliance; that is, they did not clearly distinguish chroma vision from brightness vision. Forel, however, recognized the fact that the mere ability to distinguish one chromed object from another is not a proof of chroma vision unless the objects are at the same time equally brilliant to the insect. He attempted (5, p. 181) to test the ability of bees to distinguish a given color to which they were trained from a number of grays of different brightness, but so many bees came to his dishes for food that the experiment failed.

Von Frisch (6, 7), however, carried out experiments in which this sort of procedure was successful. He set out in the vicinity of a hive a series of colored squares of paper, arranged after the manner of a checkerboard, consisting usually of one chromed paper (of the so-called Hering series), about 15 by 15 cm., and from 15 to 31 gray papers each of the same size but different in brightness, varying in approximately equal steps (for the human eye) from white to black (6). During the training period he placed on the chromed paper a watch glass containing sugar water and on each of the grays an empty watch glass. The position of the chromed paper was changed fre-

<sup>8</sup> A list of these methods, with bibliography, is given by Kuhn (21).

quently to avoid training in reference to location. After a training period of from a few hours to several days the assemblage of squares was exchanged for another with fresh papers and clean watch glasses, all of which, however, were now empty. The result was that the bees came flocking to the chromed paper and practically disregarded the grays. This was also true when none of the papers carried watch glasses, and even when the grays carried watch glasses containing food while the chromed paper carried an empty watch glass. The chromed paper must have stimulated the bees differently, then, from any of the grays which were used, and since the grays varied in brightness from black to white, Von Frisch concluded that this difference in stimulation must have been due to difference in chroma, especially since he found that the bees could not distinguish between the grays.

Severe criticism of Von Frisch's work was made by Von Hess (17), who pointed out principally the lack of consideration which Von Frisch has given to the bees' sense of smell. Von Hess went so far as to obtain samples of the different dyes used in the Hering papers and noted that some of them were distinctly different from others in odor—even to man. He made a number of experiments with these papers, in which he used a plate of glass to cover them and claims to have obtained no evidence at all of chroma vision. For example, he made a "spectrum" consisting of 185 chromed papers ranging from red to blue, in small steps, so as to give the appearance of a prismatic spectrum. After "training" (Von Hess always put "Dressur" in quotation marks) bees to yellow for several days, he set out this whole "spectrum" covered with glass, on which he made a streak of honey running down the middle of the "spectrum" for its entire length. Bees did not collect first on the yellow but came indiscriminately and collected more or less uniformly along the whole streak. Von Hess therefore flatly contradicts the conclusion that bees possess chroma vision.

Von Frisch then modified some of his experiments in an attempt to meet Von Hess's criticisms. For example, he eliminated the possibility of training to a particular odor carried by the papers, by covering the whole series with a glass plate; this precaution made no significant change in the results. He even sealed papers within glass tubes and obtained the same results. He again (7) concludes, therefore, that bees possess chroma vision.

While this conclusion of Von Frisch thus seems to be founded on sound experimental results, it is valid only if the brilliance of each of the chromed papers was, for the bees, equal to that of some one of the grays. But it is by no means certain that this condition obtains, for it is possible that the chromed papers may have been more brilliant than any of the grays, and, indeed, than even the white. This has been ably pointed out by Lutz (28), who recalls a fact stated many times by Von Hess and others, that other animals may not have the same limits of perception in regard to wave length as human beings have. That is, it is entirely possible that the chromed papers used by Von Frisch reflected ultra-violet in so much greater amounts than any of the grays that to an animal relatively highly sensitive to ultra-violet these papers would appear brighter than even the white. As a matter of fact, Lutz shows that bees and several other insects are relatively much more sensitive to ultra-violet than man—a fact that has been suspected ever since Lubbock (1) demonstrated the sensitivity of ants

to ultra-violet. Lutz then tested the Hering papers used by Von Frisch and found most of them to reflect some ultra-violet. He did not have samples of the grays to test, hence could make no comparison of the ultra-violet reflection of grays and chromas. The fact that in some experiments Von Frisch used a plate of glass to cover his papers does not entirely exclude the effect of ultra-violet, since ordinary glass often transmits down to the wave length 320  $m\mu$ .

Lutz, then, while concluding that Von Frisch is probably correct in ascribing chroma vision to bees, does maintain that he has not proved it. He said:

Hess may be right in believing that insects are totally color-blind. Probably Von Frisch is more nearly correct in saying that they can distinguish all of the colors except red and certain greens as colors \* \* \*.

Kühn (20, 22) performed some experiments designed to meet such criticisms as those made by Lutz. He made several tests, one of the simplest of which may be described as follows: Bees were trained to come for food into a semidark room and there to congregate at a narrow trough filled with sugar water and illuminated from above by a narrow streak of spectral light—e. g., from the blue-green region. After a sufficient period of training, the bees congregated on this streak of light even when the trough of food was removed. If now another streak of light of the same wave length but different intensity was thrown diagonally across this streak, after the manner of the letter X, the bees always collected on the more intense of the two streaks, no matter to which they had been trained. But when a streak of white light, taken directly from the same source as that used to produce the spectral streak, was thrown across the training streak, the bees did not collect on the white but continued to collect on the spectral streak. This he regards as proof that the spectral streak was for the bees qualitatively different from the white streak. But this is true only if the white streak was more brilliant to the bees than the other. On first thought it appears obvious that a beam of white light should always be more brilliant than any of the monochromatic constituents of that white beam. But Lubbock (7) long ago demonstrated that this does not necessarily obtain; he showed that white light after it has been modified by being passed through a certain filter is more stimulative to *Daphnia* than the same white light unfiltered. Hence it is possible that in Kühn's experiment the bees continued to flock to the spectral color merely because it was more brilliant to them than the white; in other words, it had a greater quantitative effect. From these facts it seems evident that the problem as to the presence of chroma vision in bees is open to further investigation.

#### METHODS AND RESULTS

In order to test the presence of chroma vision in bees it was decided to do three things: (1) Select a series of filters transmitting different portions of the spectrum, (2) ascertain the relative brilliance to bees of the light transmitted by each, and (3) ascertain by experiments in training whether or not bees can select a given one of these chromas from the others after all have been made equal in brilliance.

The filters selected were four made of glass and a fifth, consisting of a glass cell 3 cm. deep, filled with a solution of potassium dichromate (24 gm. per liter). The transmission curves of these filters are presented in Figure 5. The height of the peak of each curve repre-

sents its relative total transmission,<sup>9</sup> and the points of intersection of each with the base line represent with fair accuracy its limits of wave length as determined by observing through a spectroscope the lines of the mercury-vapor spectrum which it transmits,<sup>9</sup> and by the use of spectrograph photographs (10). The shapes of the various curves are only approximate, since the transmission for various wave lengths was not determined, but the shapes agree with those of curves for similar glasses, or even identical glasses but of different thickness, as given, for example, by Gibson, Tyndall, and McNicholas (13). Since all of these filters when used alone transmit a large proportion of infra-red rays, a cell containing a solution of copper sulphate (19 gm. per liter) 3 cm. deep was always used in connection with them to screen out these rays. A curve for the combined solutions of copper sulphate and potassium dichromate is given by Gibson (11, p. 274).<sup>10</sup>

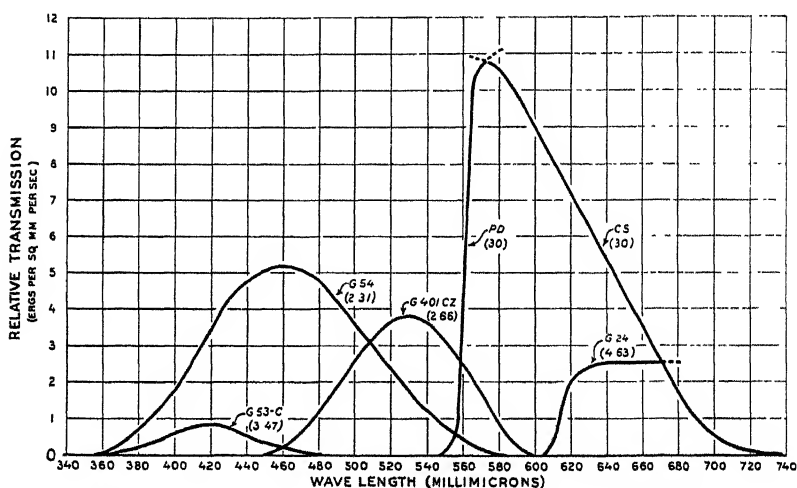


FIGURE 5.—Transmission curves for the color filters used in the study of chroma vision in bees: G53-C transmits violet; G54, blue; G401CZ, green; and G24, red; PD is the curve for potassium dichromate and CS that for copper sulphate. The dimensions in parentheses are the thicknesses (in millimeters) of the filters used

It is, of course, very difficult to find five color filters all of which transmit entirely separate and distinct portions of the spectrum. It will be observed here, for instance, that the yellow filter transmits at the same time all that the red filter does, and the blue all that the violet does. And yet these filters appear unmistakably different to the normal human eye. The reason the blue appears different from the violet is, obviously, because the major portion of the light transmitted by one is in the region we call blue and by the other in the region we call violet. If a bee can distinguish one of these from the other after they have been made equal in brilliance to the bee, we may say with confidence that the bee possesses chroma vision, for even though the transmission curves overlap, whatever difference there is, under these conditions, must be due to chroma.

<sup>9</sup> Kindly determined for the writer by R. Stair in the laboratory of W. W. Coblentz.

<sup>10</sup> The curve in the reference cited is for a solution of copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) containing 57.0 gm. per liter and for a solution of potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) containing 72.0 gm. per liter, each in a cell 10 mm. thick. Since in the present investigation the glass cells were 3 cm. thick, the concentration was reduced by two-thirds, giving, according to information personally furnished the writer by Gibson, the same curve as that shown in the reference.

The chromas having been selected, the next problem was to find their relative stimulative effect (relative brilliance) for the bees. One of the main difficulties in doing this proved to be the finding of a suitable reaction to measure—by no means an easy task. It was found, for instance, that when the bees were placed in the intersection of two wide beams of light their reactions could not be measured accurately because of the bees' excitability and rapid movements. Likewise, the ratio of the numbers that congregated at the two ends of a box into each end of which a beam of light entered—although this method was used by Von Hess (18) with apparent success—was found in these experiments not to be a sufficiently delicate measure of the relative brilliance of the respective beams, because of the great restlessness of the bees and the ready modification of their behavior through inability to escape, and other incidental factors.

A much more satisfactory measure of the relative stimulative effect was found in the reactions of individual bees to two beams of light coming from opposite directions, as these reactions

were observed in the apparatus now to be described. (Fig. 6.) A beam of light from a 400-watt projection lamp passes first through a solution of copper sulphate (to remove the infra-red rays) and then through a pair of rotating sectors, one variable in position with respect to the other. (Fig. 7.) The upper half of the beam is intercepted by only the larger of the two rotating disks *d* but the lower half is intercepted by both. When the sectors of the two disks are made to coincide with each other and are then rotated together, the light intensity of the lower half and that of the upper half of the beam are identical; but by turning the smaller disk *c* with respect to the larger and rotating them together the intensity of the lower half of the beam can be diminished to any desired degree until, when the sectors are exactly opposite in position, it is completely screened out. The position of the smaller disk with respect to the larger can be varied by means of a suitable knurled collar, graduated in percentage.<sup>11</sup> After passing the sectors (fig. 6), the upper half of the beam is deflected by a mirror and proceeds at an angle of 90° to its former direction, while the lower half of the beam is allowed to pass on. The two beams, now proceeding in directions at right angles to each other, are caught at equal distances from the first mirror by other mirrors which turn

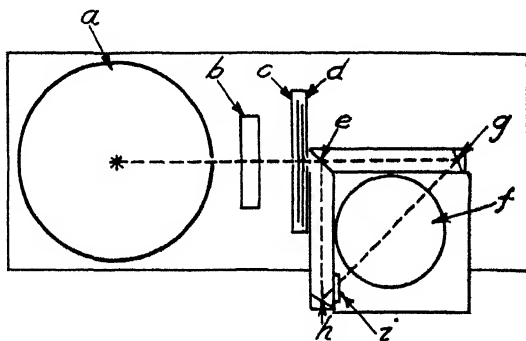


FIGURE 6.—Explanatory diagram of apparatus used to ascertain the relative stimulative effect on honeybees of transmitted light of various chromas: *a*, 400-watt lamp, the source of illumination; *b*, glass cell containing a solution of copper sulphate, for screening out infra-red rays; *c*, *d*, two rotating sectors, adjustable with respect to each other, for transmitting two beams of light of variable relative intensity; *e*, mirror for reflecting one of the two beams at a right angle; *f*, cover (inverted) of a large Petri dish, for containing bees under observation; *g*, *h*, mirrors for reflecting the two beams into two opposite sides of the inclosure; *i*, light filter

<sup>11</sup> The rotating sectors and the lamp house of this apparatus are a part of the Keuffel and Esser color analyzer.

them directly toward each other. The beams now pass through light filters, if desired, and enter the opposite sides of an inverted cover of a large Petri dish, under which are imprisoned from one to three bees. Since the apparatus is set up in a dark room, with the lamp house well shielded, these two beams are practically the only light which strikes the bees. Figure 8, from a photograph, shows the apparatus as a whole, except the Petri dish and the top of the lamp house.

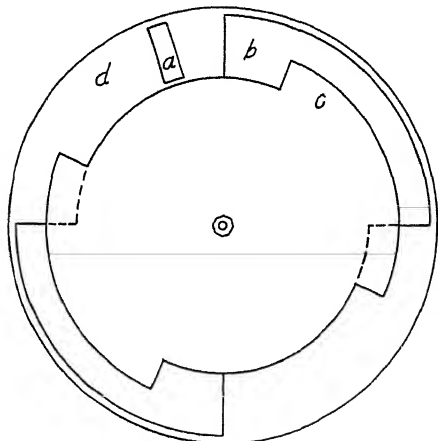


FIGURE 7.—Diagram of rotating sectors used to vary the relative intensity of the upper and lower halves of a beam of light: *a*, The aperture through which the beam passes; *b*, the larger rotating disk, which always intercepts the whole beam, reducing its intensity by one-half; *c*, the smaller rotating disk, its position variable with respect to the other, which intercepts only the lower half of the beam of light, reducing its intensity to any additional degree desired, according to its position with respect to the larger disk; *d*, the housing which incloses the disks

It was found that under these conditions the bees walk hurriedly around under the glass dish, and whenever they come to the spot on the side where a beam of light enters they pause and start as if to walk up the side of the dish. After an attempt or two they pass on until they come again to a spot where a beam enters. When the two beams are decidedly unequal in intensity the bees attempt to escape most often at the more

intense beam; by increasing the intensity of the weaker beam, however, a point is reached at which this beam induces about as many of

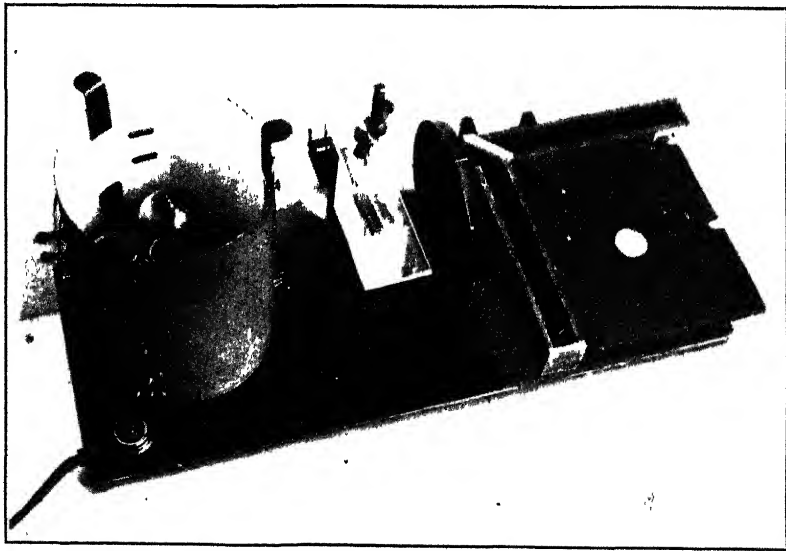


FIGURE 8.—General view, from a photograph, of the apparatus illustrated in Figure 6. The Petri dish and the top of the lamp house are lacking

these peculiar reactions as the other. This is then considered the point at which the two are equal in brilliance for the bee.

The procedure, then, was first to place one of the chromed<sup>12</sup> filters, mentioned above in the text and in connection with Figure 5, in the constant beam, leaving the variable beam white. A count of the reactions to each then indicated whether the chromed or the white beam was the more stimulative to the bees. If the white was the more stimulative its intensity was reduced, if the less stimulative, it was increased, after which, in either case, a count was again made. In this way, after making a series of tests, a percentage of the total white available could be found which induced approximately the same number of reactions as a given chromed beam. This percentage, obtained for each filter, was regarded as a representation of the relative stimulative effect (relative brilliance) of the light produced by each filter.

It was found that the exact percentage of white required to equalize a given chromed beam in stimulative effect was very difficult to ascertain accurately. The difficulty may have been due to an insufficiently delicate means of measuring the reactions, but it was probably due rather to an inability of the bees themselves to recognize differences in brilliance between a chromed and a white beam unless those differences are of considerable magnitude.

In the method finally chosen for finding these values the percentage of available white used was first made so low that in most tests it definitely induced fewer reactions than the chromed beam. Then the intensity of the white was increased by small steps until it definitely induced more reactions than the chromed beam. By putting down in order the results of these tests, a point could be chosen which seemed to represent with fair accuracy the percentage of white which just equalled the chroma in stimulative effect. Thus, in the experiment in which green was compared with white, white at the intensity of 15 per cent of the total white available induced 48 per cent of the total reactions; at the intensity of 20 per cent it induced 45 per cent of the total reactions; and with the percentage of white increased at suitably chosen steps, fully set forth in Table 4, the percentage induced of the total reactions increased correspondingly to 58 per cent, both for 40 and for 56 per cent of the maximum available white. In this series it appears that any percentage of white between 15 per cent, or perhaps lower, and 35 per cent was approximately as stimulative to the bees as green. There is an indication of a gradual rise, however, so that the most probable value for equal stimulative effect seems to fall at from 28 to 30 per cent, and the latter figure was adopted.

These results and those obtained for the other chromas are presented in Table 4. The percentage of white required to equal the effect of blue seemed to lie between 7 and 25, with the mean at about 20, and for the yellow the percentage seemed to lie between 15 and 30, with the mean also at about 20. For the violet and red the brilliance was very low and hard to measure. For the former the mean percentage of white required was about 2, and for the latter between one-fourth and one-half, and the larger fraction was chosen.

<sup>12</sup> In accordance with the definition of chroma discussed at the beginning of Part II, a chromed filter, chromed beam, etc., is any filter, beam, etc., whose color is some other than a shade of gray.



TABLE 4.—Summary of experiments performed to find the relative percentage of white light required to equal in stimulative effect the light transmitted by five light filters

Color of filter	Available white used	Bees used	Reactions	Reactions induced by white light	Color of filter	Available white used	Bees used	Reactions	Reactions induced by white light
	Percent	Number	Number	Percent		Percent	Number	Number	Percent
Green	15	6	500	48	Yellow	15	5	600	51
	20	5	150	45		20	6	500	47
	25	2	150	47		25	10	1,200	51
	28	4	300	18		30	4	550	53
	30	5	500	51		35	6	650	53
	35	10	750	51		40	4	350	52
	40	2	50	58	Violet	50	2	150	59
	50	2	50	58		1	8	350	11
	7	3	200	49		2	10	725	55
	10	3	200	50		5	1	50	66
Blue	15	9	550	50		11	2	25	28
	20	3	500	49	Red	12	8	275	62
	25	9	825	50		1	2	50	82
	30	4	350	55		7	2	25	81
	35	2	200	52					
	48	3	100	56					

a Chosen as being equal to the chromed light in each series.

Multiplying these percentages by 2 to eliminate the fraction, we may say that the relative brilliancy of these chromas for bees stands approximately in the numerical ratio of green 60, blue 40, yellow 40, violet 4, and red 1, with the understanding that these are merely mean values. They do not represent the relative efficiency of the chromas considered, but rather the relative stimulative effect of the chromas on untrained bees.

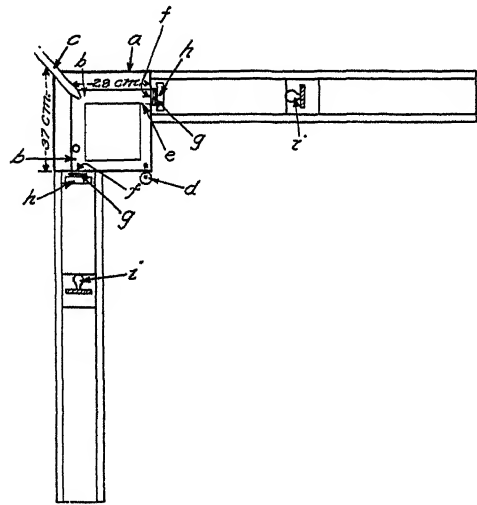


FIGURE 9.—Diagram of apparatus for testing the ability of bees to distinguish between chromas: a, Box 37 cm. square; b, b, glass-covered tunnels, about 3 cm. wide, by 2 cm. deep, within and along the sides of a square 28 by 28 cm. outside; c, passage between tunnels and exterior of building, for entrance and exit of bees; d, feeder; e, wire screen; f, f, diffusion screens of ground glass, each covering a rectangular aperture 15 by 30 mm. in size; g, g, light filters; h, h, glass cells for holding solution of copper sulphate; i, i, 100-watt electric bulbs, for illuminating apertures covered by the diffusion screens, adjustable for distance by means of carriers sliding on tracks

After these values had been found, the next procedure was to ascertain, by training and testing, whether or not bees can select any given one of these chromas from any other one when the two are made equal in brilliancy; that is, equal in equivalent percentages of the available white light used with the apparatus just described.

The apparatus (fig. 9) used for this purpose consisted essentially of a square box, measuring 37 cm. on each side, with a passage for the entrance and exit of bees in one corner and a feeder in the opposite corner. Two glass-covered passageways led from the entrance in opposite

directions, leaving at right angles to each other, and following the sides of the box around to the feeder. At a point in each of the two opposite sides of the box, visible from the entrance, a rectangular opening 15 by 30 mm. was made, through which a beam of light entered from a 100-watt lamp placed in a covered tunnel outside. From the inner end of the entrance the two openings were visible in directions at right angles to each other. The intensity of either beam could be varied by changing the distance of the corresponding lamp. A ground-glass diffusion screen was placed over each rectangular opening; as the openings were immovable and constantly equal in size and in distance from the inner end of the entrance, any movement of the lamps caused no variation in the size of the images. The chromed filters were placed just back of the diffusion screens. The whole apparatus was placed in a dark room, and only a narrow tunnel connected it with the exterior. Enough light came from the lamps through the light filters and diffusion screens to enable an observer to watch the bees in the tunnels near the entrance.

The first step in using the apparatus was to get the bees voluntarily to enter the box. This required considerable patience but offered no great difficulties. A dish of sugar water was placed out, preferably in the early morning, until it was found by bees. The first visitor, on her return to the hive, stimulated a number of her companions to rush out in all directions, and some of them came to the food. On their return to the hive, they stimulated others, and in a short time a large squad was built up, the members of which made regular trips between feeding dish and hive. Now the dish was moved toward the training box, at first only a few centimeters at a time, then, as the bees became accustomed to searching (apparently) for it, several paces at a time, letting it remain in each new location until most of the bees had come to it there. When, finally, the entrance leading to the training box was reached, drops of sugar water were placed consecutively every few centimeters along the circuitous route leading from the exterior to the feeder in the far corner of the training box. As soon as the bees had removed one drop they proceeded to the next, until finally a few succeeded in reaching the feeder. In doing this they had, first, to go from bright daylight into a dark tunnel leading from the outside the box itself, located in the dark room, then turn obliquely and walk down a brightly lighted passageway toward one lamp or the other, then turn at right angles into a dark passageway, and finally reach the feeder in the corner. The return trip involved the reverse of this procedure. This series of reactions is much more complex than the reactions used in taking nectar from a flower under natural conditions, and represents, evidently, a considerable modification in behavior. In other words, the bees following this route were learning a new set of facts, differing considerably from those of their usual experience in the hive and in the open air.

Under these circumstances the few bees that first learned the way to the feeder appeared to stimulate their companions, so that, if the food was abundant, the squad of bees became, after a few hours, so large that it was impossible to count them. And not only that, but they developed the so-called "robbing fever," under the influence of which they seemed to lose their usual well-marked ability to learn; instead, they scrambled over one another in what resembled a frenzy of hit-or-miss efforts to find the feeder. To prevent the coming of

such large numbers, one of the most successful methods found<sup>13</sup> was to reduce the size of the opening of the feeder to such dimensions that only one bee could feed at a time. The feeder devised for this purpose is diagramed in Figure 10. The sugar sirup in the inverted bottle *a* runs down through the tube *b* into the cup *c*. From here the sirup runs around through the smaller tube *d* to the small feeding cup *e* until both cups *c* and *e* are about half full. As soon as the level of the sirup in *e*, and consequently in *c*, rises to such a height that the lower end of tube *b* is closed, the entrance of any more air into tube *b* is prevented, and consequently no more sirup runs out of bottle *a*. As soon, however, as a bee feeding at the cup *e* reduces the level of the sirup in *e* and *c* to a point where more air can enter tube *b*, more sirup

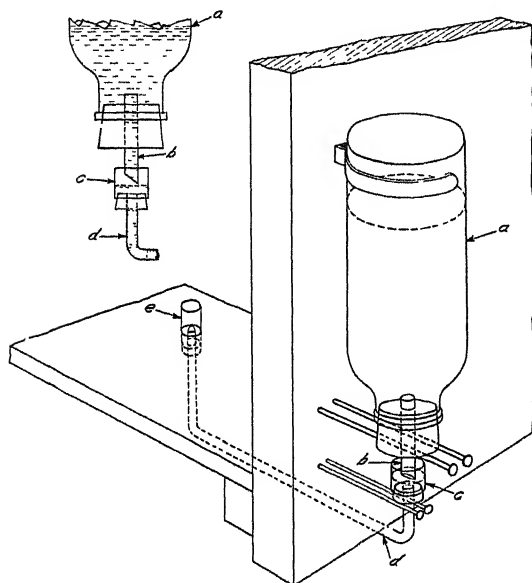


FIGURE 10.—Feeder used in apparatus for testing the ability of bees to distinguish between chromas: *a*, inverted bottle used as reservoir for sugar sirup; *b*, glass tube conducting sirup into open cup *c*; *d*, glass tube connecting *c* and *e*, the sirup in the two being always at the same level; *e*, feeding cup, made of 1/4-inch glass tubing, admitting the head and thorax of one bee at a time.

runs down from bottle *a* and the level in *e* and *c* rises again. Thus, as long as there is sirup in bottle *a*, there will be a supply also at the cup *e*, and it will be fed down only as fast as it is taken away by the bees.

The particular point of interest here is the fact that the cup *e* is large enough to admit the head and thorax of only one bee at a time. This, of course, was an effectual check on the rate at which a squad of bees could obtain food. By the aid of this contrivance and by using a suitable concentration of sugar sirup the rate at which bees came could be reduced almost as much as desired, because the time

required for a bee to feed increases with increased concentration. The sirup used in these experiments contained about 2 parts of sugar to 1 of water (by volume). With this arrangement a small squad of bees, usually not more than 15, made regular trips from the hives to the feeder and back, entering and leaving the box at the rate of about one bee a minute. There was an appearance of eagerness and purpose in their behavior, but no frenzy. Usually there were five or so crowding around at all times, ready to enter the opening of the feeder. The bees were at first marked on the back with a mixture of orange G in shellac in order to make it possible to identify the trained bees, but this precaution was found to be superfluous because very few novices came to the box, and these could be distinguished at once by their hesitation and lack of orientation. How-

<sup>13</sup> Suggested to the writer by Jas. I. Hambleton.

ever, the few novices usually learned the route after a while, and their number balanced, approximately, the number that died, so that the squad continued roughly uniform in size.

After a regular squad had been formed and its members were able to find the way to the feeder, a chromed filter was placed behind each diffusion screen and the distance of each lamp from the corresponding screen was so adjusted that the two beams had the same brilliance, according to the values already obtained and recorded in Table 4, and the training then began. Suppose it is desired to train the bees to associate with food the beam of light at the left, as the reader looks at Figure 9. The food is to be obtained only at the feeder in the corner of the box opposite the entrance. In order to reach it the bee, on entering, must walk for half the distance down a passageway directly toward one lamp or the other, then make a right-angled turn and walk an equal distance through a dark passageway until the feeder is reached. But a wire screen is placed in the dark portion of the passageway at the right, so that the bee can finally reach the food only by taking the other passage. After experiencing failure by way of the right but success by way of the left for a period of a day or two, most of the bees take the correct route without hesitation immediately on entering the box. It may be observed that the opening of the feeder is placed in the corner of the box opposite the entrance, so that any odor emanating from the food will be equally present in both passages.

Out of a total number of bees observed as they enter the box, the number taking the correct route—i. e., the route leading to the food—gives the "total per cent trained." They are presumably trained to position, but they may or may not be trained to chroma; that is what is now to be ascertained. To do this the position of the two beams of light is exactly reversed by interchanging the filters and adjusting the distances of the lamps accordingly, but the wire screen is left unchanged. The percentage of the original total number continuing to take the route leading to the feeder after this reversal gives the "per cent trained to position only;" that is, they do not follow the training chroma but continue to go to the old location; they are trained to position rather than chroma. Subtracting the "per cent trained to position only" from the "total per cent trained," we obtain the "per cent trained to chroma."

An example will make the procedure clear: Date, October 21, 1927; training chroma (at left), blue; opposite chroma, violet; length of training period, four hours. At the end of these four hours of training the observer took his stand at the entrance to the box and watched the reactions of 10 bees as they entered. Of these, 8 turned at once toward the blue (i. e., toward the left) and went directly around to the feeder in the far corner. Hence the "total per cent trained" is 80. Now the chromas were interchanged, blue at the right, violet at the left, wire screen still at the right, and the next 10 bees that entered were observed. Of these, 5 turned to the left as the 8 had done before, although now the color at the left was violet instead of blue. Evidently these 5 were not trained to chroma, but to position only; hence the "per cent trained to position only" is 50. Subtracting this latter figure from 80, the "total per cent trained," we obtain 30 as the "per cent trained to chroma."

This method of calculating the net percentage trained eliminates the possibility of including bees which are trained merely to location and not to chroma. It is of course apparent that in this apparatus the bees depend in the training process not only on their sense of sight but also on their sense of direction; that is, they become trained to turn in a *right or left direction* as well as toward a given quality of light. Hence, after a training period, when the position of the two chromed beams is exchanged, no bees will follow the training chroma to its new location unless the association with light is stronger for them than the association with direction. The values obtained, then, for percentage trained to chroma will always represent the training to light which exceeded the training to direction. For this reason, even small percentages of training to chroma are to be regarded as significant.

The results of the 23 experiments performed are presented in Table 5. It will be observed, in the column "Per cent trained to chroma," that in every experiment except three there was distinct selection of the chroma associated with the food (the training chroma). That is, after being trained to blue, for example, in the pair blue versus violet, the bees followed this blue when its position was changed, although as far as its nonchromatic brilliance was concerned it must have been indistinguishable to them from the violet. Selection occurred irrespective of which chroma of the pair was used as the training chroma—i. e., as the chroma toward which the bees had to travel during the training period in order to reach the feeder.

TABLE 5.—Summary of results of a series of experiments performed to test the ability of bees, after they had been trained to associate color with food, to distinguish between chromas of equal stimulative influence

Training chroma	Opposite chroma	Period trained	Total trained	Trained to position only	Trained to chroma	Average trained to chroma
		Hours	Per cent	Per cent	Per cent	Per cent
Blue	Violet	4	80	50	30	25
		10 <sup>1</sup> / <sub>4</sub>	80	60	20	
		11 <sup>1</sup> / <sub>2</sub>	60	0	60	
Do	Green	2 <sup>1</sup> / <sub>2</sub>	80	60	20	32
		4	80	40	40	
		6	70	50	20	
Green	Blue	6	70	50	20	40
		10 <sup>1</sup> / <sub>2</sub>	100	60	40	
		7	80	50	40	
Blue	Yellow	8 <sup>1</sup> / <sub>3</sub>	100	87	13	16.5
		9 <sup>5</sup> / <sub>4</sub>	90	70	20	
		8 <sup>1</sup> / <sub>2</sub>	100	60	40	
Red	Blue	8 <sup>1</sup> / <sub>2</sub>	80	30	50	45
		20	80	70	10	
		11 <sup>1</sup> / <sub>2</sub>	80	50	30	
Green	Red	8 <sup>3</sup> / <sub>4</sub>	70	90	-20	20
		12 <sup>1</sup> / <sub>2</sub>	70	70	0	
		8 <sup>3</sup> / <sub>4</sub>	70	40	30	
Red	Green	9	90	50	40	12.5
		30	80	80	0	
		40	90	80	10	
Yellow	do	17	80	60	20	17.5
		8	90	50	40	

\* In nearly all experiments the percentages given in these two columns are based on observation of the reactions of 10 bees. For instance, 80 per cent means that 8 of out 10 bees reacted as indicated.

Of the three experiments tried in which selection did not occur, two were in the series in which the pair red versus green was used and one in the case of yellow versus green. These three experiments

gave a net percentage trained of minus 20, 0, and 0, respectively, minus 20 meaning, of course, that more bees went in the direction that led to food after the chromas were interchanged than went in that direction before. It seems, however, that no particular significance is to be attached to these values (which simply indicate complete lack of training to chroma) because the other experiments in the same series, performed on the next few succeeding days, gave higher values, from 10 to 40 per cent. It is probable, therefore, that these low values are merely accidental variations.

Do these results definitely establish the presence of chroma vision in honeybees? By definition, chroma vision is the ability to distinguish between diverse chromas of the same brilliance. There can be no question as to the ability of bees to distinguish between the chromas used in the experiments just described. (Table 5.) The only objection that can be raised is with reference to the values for stimulative effect of these chromas, found as described above and presented in Table 4. That is, if, perchance, owing to some fault in these tests, the relative brilliance of blue and violet, for example, were not really in the ratio of 40:4 (Table 4) but, say, 30:4, then their equalization on the basis of 40:4 would have left them really different in brilliance and the selection exhibited by the bees could have occurred on the basis of brilliance and not of chroma. But if it were true that the bees selected these chromas on the basis of brilliance alone, then, by varying in both directions the intensity of one member of any given pair of chromas, one ought to find somewhere an intensity at which its brilliance is just equal to that of the other chroma of the pair and produce thus a condition in which no selection would occur.

A series of experiments of this kind was performed, using the chromas yellow and green, which, according to Table 4, have a relative brilliance of 20:30. In the first experiments the chromas were equalized on this basis (lamps set 32.6 cm. from yellow filter, 40 cm. from green filter) and gave in four experiments percentages of bees trained to chroma of 0, 10, 20, and 40, having an average of 17.5. Then the intensity of the yellow was reduced to 75 per cent of its previous value (distance of lamp increased to 37.7 cm.), and two experiments gave percentages of 20 and 60, or an average of 40. Then the intensity of the yellow was still further reduced, to 44 per cent of its original intensity (distance of lamp increased to 49 cm.), and two experiments gave percentages of 40 and 50, or an average of 45. Finally, the intensity of the yellow was increased to 125 per cent of its original intensity (distance of lamp decreased to 29.2 cm.) and three experiments gave percentages of 15, 10, and 23, or an average of 16. Hence, even though one refuses to accept the chosen stimulative values, in terms of white light, of the chromas considered in Table 4 as accurate, and uses instead various other relative intensities, the result is the same, within the purpose of this research; the bees continue to distinguish between the chromas.

It is therefore maintained that the presence of chroma vision in bees is established.

#### THE NUMBER OF CHROMAS DISTINGUISHABLE BY BEES

It is interesting to observe, in passing, some work that has been done on the number of different chromas bees can distinguish and the bearing of this work on the problem of the experiments recorded

in this paper. Von Frisch's (6, 7) experiments in training bees to come for food to various colored papers led him to conclude that they are able to distinguish only two chromas, the short-wave region comprising the blue and violet and the long-wave region comprising the green, yellow, and light red, and that they are chroma blind to blue-green and dark red. Kühn and Pohl (24), using a similar method, but with sections of the mercury-vapor spectrum instead of colored papers, concluded that there are four regions of the spectrum distinguishable by bees—the yellow and green region, the blue-green region, the blue and violet region, and the ultra-violet region. Kühn (22), supplementing the mercury-vapor spectrum with the continuous spectrum from a carbon arc, defines these regions more precisely as having for limits the wave lengths 650 to 510  $m\mu$  for the red, yellow, and green region, 500 to 480  $m\mu$  for the blue-green region, 480 to 400  $m\mu$  for the blue-violet region, and 400 to 313  $m\mu$  for the ultra-violet region.

On the basis of the experiments recorded in the preceding pages, the writer (4) in 1928 contended that bees are also able to distinguish blue from violet and green from light red. In Table 5 of the present paper, for example, blue is shown to be distinguished from violet by 25 per cent of the bees, and green from red by 20 per cent in one group of experiments and 12.5 per cent in another. But the width of the band of spectrum transmitted by the filters used, as shown in Figure 5, prevented him from giving the proof for such a contention, and therefore in writing the present paper no point was made of it. That is, the blue might have been distinguished by the bees from the violet because of the amount of blue-green transmitted by the blue screen, and the green may have been distinguished from the red because of the amount of blue-green transmitted by the green screen. If the contention is true, however, there are altogether six chromas which a bee can distinguish.

But subsequent to the appearance of the paper (4) cited in the preceding paragraph, and also subsequent to the writing of the first part of the present paper, there has come to the writer's attention a short article by Kühn and Fraenkel (23), in which they state that further work along the line pursued by Kühn (22) has shown that in the region comprising red, yellow, and green, and again in that comprising blue and violet, there are three nuances of color qualities distinguishable by bees. Hence the total number of chromas which it is claimed bees can distinguish now stands at eight.

One wonders if further research will not disclose still other chromas in the spectrum which are visible to the honeybee. By way of comparison it is interesting to note that for the human eye, according to Nutting (32, p. 60-61), there are "from about 130 to 180 distinctly different pure hues between the red and violet, while from violet back to red through the purples and magentas there are about 20 additional steps as determined by their complementaries in the green."

#### CONCLUSION

In view of the findings of the research described under "Methods and results" in the present article, it is maintained that the presence of chroma vision in bees is established, and that the conclusion of Lubbock, Von Frisch, Kühn, and others is confirmed. The very fact of chroma vision connotes a diversity of chromas distinguishable by

bees, several of which have been reported by investigators, and it is presumable that the number of such distinct chromas will be augmented by further research.

### III. ABILITY OF THE HONEYBEE TO DISTINGUISH DIFFERENCES OF BRIGHTNESS

It seems self-evident that any animal that sees at all must have some ability to distinguish differences in brightness. Particularly ought this to be true for the honeybee, which, as is well known, reacts strongly to light. For this reason one is surprised to find in the literature records of experiments in which it is indicated that bees are entirely unable to distinguish any except relatively enormous differences in brightness. Von Frisch (7, *p. 105*), for example, after arranging in the manner of a checkerboard a series of pieces of paper colored various shades of gray, trained bees to come for food to a given one of these papers; then he rearranged the papers in different order and took the food away to see if the bees would seek out the same shade of gray as that on which the food had previously been placed. But they did not; they went to all the grays. Only when he used a very dark gray or a very light gray for training did the bees exhibit any evidence of ability to pick out the corresponding shade. He concluded, therefore, that bees possess a very poorly developed sense of brightness.

Von Hess (18), on the contrary, using a method in which training of the bees was not involved, concluded that they have practically as acute a sense of brightness as does man. In one experiment, for example, he confined bees in a cubical glass box on two opposite sides of which were placed tunnels containing lamps, one of which could be moved within its tunnel nearer to or farther from the box. Between the tunnel and the glass box on each side was placed a piece of white paper to serve as a diffusion screen. When the intensity of the light on the two paper screens was very unequal the bees crowded together toward the brighter, when equal they distributed themselves at random throughout the box. Von Hess observed that when the intensities on the two screens were changed from equality to a ratio of 1.00:0.86 they began to appear different in brightness to his own eyes, and that when the ratio was changed slightly to 1.00:0.83 the bees began to distribute themselves unequally in their container. Other experiments gave similar results, indicating that the bee is only slightly less acute than man in distinguishing differences in brightness.

Kühn (20), however, came to a conclusion agreeing with that of Von Frisch. He trained bees to come for food to a narrow trough illuminated by a streak of white light of a given intensity. Then, after they had become well trained, he removed the trough and threw on the table with the original streak of light another streak of different intensity which crossed the former diagonally, the two streaks together thus making a figure like the letter X. When the bees now came searching for food they alighted on the more intense of the two streaks, no matter which was the one used in their training. Kühn (20, *p. 118*) concludes, "Auf eine bestimmte Helligkeit sind die Bienen in diesen Versuchen nicht dressiert und auch nicht dressierbar."



The writer has had occasion to use both the method involving training and that involving merely untrained reactions. By use of the former method he (4) obtained evidence which led him to conclude that bees are "trainable" to differences in brightness. Using the same apparatus as that employed in testing chroma vision, described in Part II of this paper (fig. 9), but without the color filters, and the same method of procedure as described there, he was able to show that bees can distinguish two illuminated areas when one is as much as, or more than, 4 times as bright as the other, but probably not when one is only 1.3 times as bright as the other.

By use of a method involving only the untrained (instinctive) reactions of bees, the writer also demonstrated their ability to distinguish differences in brightness. The method used is described in the experiments dealt with in Part I of this paper. Bees were placed at one end of a dark box and allowed to walk toward two illuminated areas situated about 10 cm. apart at the opposite end of the box. In some experiments the illuminations of these areas were made equal in intensity and in others unequal in a variety of widely separated proportions. It was found, in brief (Table 2), that when the intensity of one was decreased (by large intervals) the relative number of bees going to that one decreased also, not in direct proportion but according to a curve (fig. 2) discussed there and of no particular interest here. That set of experiments did, however, establish a method of investigating this problem and confirmed Von Hess's conclusion to the extent that bees do instinctively distinguish between the brightness of two illuminated areas provided they are sufficiently different in brightness, but it did not show the *least* difference in brightness that bees can distinguish. (The ratio given in Table 2 which represents unequal intensities having the smallest difference in brightness is 0.50, which means, of course, that one intensity was half as great as the other.)

What is evidently needed, therefore, is a series of experiments to test in a quantitative way the bees' ability to distinguish differences in brightness.

Moreover, since at the present time there is much interest in comparisons of the acuity of various senses in bees with that of the corresponding senses in human beings, it also seems valuable to make another test of the relative acuity of the sense of brightness in these two. It was commonly believed in former times that the bee's senses are remarkably acute, but most of the results of actual tests have shown that they are less acute than those of man. As to taste, for example, Von Frisch (9) finds that the bee appears to be less acute than man for sweet and for bitter; and as to smell (8) it is said to be no more acute than man. In regard to sight, bees are far less able to distinguish chromas than we are, there being only eight chromas distinguishable by bees, according to Kühn and Fraenkel (23), in contrast to the 150 or so distinguishable by man; and in ability to distinguish details of shape and form they are far less acute than man (2, 16); according to Hecht (15) they are about one one-hundredth as acute. The question thus persists as to how bees compare with human beings in the ability to distinguish differences in brightness. Are they very much inferior to man, as Von Frisch and Kühn maintain, or practically equal to man, as Von Hess maintains? Although there is available much information on this subject in regard to human

beings, as for example that reported by Nutting (32), it can not, in fairness to the bees, be used in this comparison because it has been obtained with much more refined apparatus than can be used with bees.

A series of experiments was made, therefore, to obtain with the same apparatus for both bees and human beings as accurate quantitative measurements as possible of their ability to distinguish differences in brightness. These experiments are now to be described.

#### METHOD

The apparatus (fig. 11) was essentially the same as that used in a previous series of experiments (Part I). It consisted of a shallow, rectangular box, *a*, covered with wire screen, 67 by 26 by 4 cm. inside, and of three boxes, *b*, *c*, and *d*, for holding lamps. The smallest lamp box, *b*, was placed at one end, designated as the left-hand end, of the large box *a*, the two others at the opposite or right-hand end. Light from the lamp in box *b* illuminated a small area of a ground-glass screen *e* placed in the center of the left-hand end of the large box *a*, and the lamps in boxes *c* and *d* illuminated areas 6.5 by 13 mm. on the

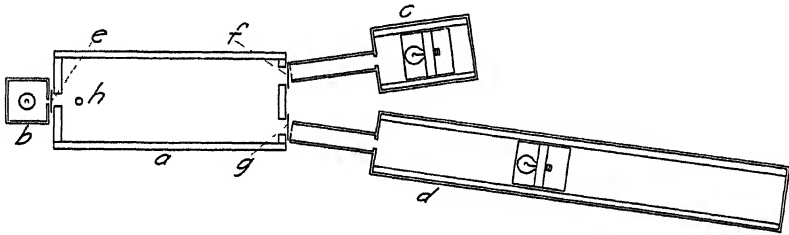


FIGURE 11.—Diagram of apparatus used in studying the ability of bees and of human beings to distinguish between small differences of brightness. See text for explanation

ground-glass screens *f* and *g*, placed equidistant from the corners at the right-hand end of the large box *a*. A hole in the floor of the large box in the mid-line near the left-hand end permitted the entrance of bees. The whole apparatus was placed in a dark room. Box *b* contained a 15-watt lamp, the boxes *c* and *d* either 10-watt or 75-watt lamps, always both the same in any one test. The lamp in box *c* remained always at the constant distance of 40 cm. from the screen *f*; the lamp in box *d* was placed either at 40 cm. from screen *g*, where the intensity on the screen was equal to that produced by the lamp in box *c*, or at 42.1 cm., 44.7 cm., 48.0 cm., 51.6 cm., 56.0 cm., 63.2 cm., or 73.0 cm., where the intensity was reduced, respectively, to 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, or 0.3 the value of that produced by the lamp in box *c*. The lamps in boxes *c* and *d* were interchanged at the middle of every experiment to neutralize any possible difference in brightness between the two lamps. The lengths of the wires leading from the switch to the lamp sockets in *c* and *d* were made equal, in order to obviate any difference in brightness due to differences in resistance.

In performing an experiment with this apparatus, a bee was captured at a feeder,<sup>14</sup> in a small screen-wire cylinder about the size of an ordi-

<sup>14</sup> Field bees must be used. Hive bees that have not yet begun to collect from the field are not positive to light.

nary test tube, taken inside the dark room, and fed with sugar water (by inverting over the screen-wire cylinder a small vial containing this liquid, its mouth covered with a layer of cheesecloth) until it was satiated,<sup>15</sup> then it was released into the large box *a* through the hole *h* in the floor. At the same time the lamps in boxes *c* and *d* were turned on, so that the bee on entering found itself confronted by two illuminated areas 60 cm. away and 10½ cm. apart. Sufficient light diffused from the illuminated areas to enable the observer to see the bee in the box. The bee now walked toward the spots of light, soon veering toward one or the other, and eventually bumping into one of them if allowed to proceed far enough. Ordinarily, however, as soon as it could be ascertained which lighted area the bee was headed for, the lamps in boxes *c* and *d* were turned off and that in box *b* turned on, and the bee immediately turned around and walked toward the source of light in the latter box. When it had come near the hole in the floor through which it had entered, the lamp in *b* was suddenly turned off and the lamps in *c* and *d* turned on, whereupon the bee turned around and walked again toward the right-hand end of the box, going toward one or the other of the areas illuminated by the lamps in *c* and *d*.

This marching and countermarching was continued until, for most of the tests, the bee had made five trips toward the right-hand end of the box. Detailed records were kept as to which area it went on each trip. Such a record, giving the responses of 20 bees to areas whose intensities of illumination had the ratio of 0.3:1.0, is presented in Table 6. In the summarized results records were made (1) of the total responses of all bees to each pair of illuminations and (2) of the sum of the initial responses of individual bees to a pair of illuminations. It was thought that the initial response of the bee when first faced with these two illuminated areas might better indicate which area really appeared the brighter to the bee than the sum of five reactions, but this idea does not appear to be correct.

After one bee had made five successive trips it was taken out into the open and released and another bee captured and given the same treatment. Experience had previously shown that after five such trips many bees become sluggish, and begin to wander aimlessly about the box, apparently having lost much of their positiveness to light.

For most of the tests a total of 30 bees were used for each pair of illuminations, giving therefore 150 responses. For some pairs of illuminations data previously gathered were included, giving as many as 1,150 responses for a single pair. Eight pairs of illuminations were employed, the ratios of intensities of which have been given, with two sizes of lamps, making 16 tests in all.

<sup>15</sup> The feeding tends to quiet the bees and prevent them from racing wildly over the box when released into it.

TABLE 6.—Detailed record of two tests, giving reactions of bees to a pair of illuminations having intensities in the ratio 0.3:1.0<sup>a</sup>

[Left-hand lamp 10-watt, at a distance of 40 cm ; right-hand lamp 10-watt, at a distance of 73 cm.]

Date August, 1928	Hour	Bee No.	Reactions	Date August, 1928	Hour	Bee No.	Reactions
7	10 30	1851	. . .	7	3 35	1871	. . .
7	10 40	1852	. . .	7	3 40	1872	. . .
7	10 45	1853	. . .	7	3 45	1873	. . .
7	10 50	1854	. . .	7	3 50	1874	. . .
7	11 00	1855	. . .	8	9 30	1875	. . .
7	11 05	1856	. . .	8	9 35	1876	. . .
7	11 10	1857	. . .	8	9 40	1877	. . .
7	11 15	1858	. . .	8	9 45	1878	. . .
7	11 35	1859	. . .	8	9 50	1879	. . .
7	11 45	1860	. . .	8	9 55	1880	. . .
			24 26				38 12

<sup>a</sup> This table is an attempt to reproduce as exactly as is practicable in print a small part of the writer's record of the observations described in the text. Each bee used was assigned a number, to the right of which a series of five dots, reading downward, represents the five consecutive reactions of the bee. A dot to the left of the vertical line records the approach of the bee to the ground glass *f* (fig. 11), and one to the right of that line an approach to the ground glass *g*, and vice versa. The hours indicated in this table range from 9.30 a. m. to 3.50 p. m.

For comparison of the acuity of vision of the bee with that of man it is evident that the same or similar apparatus must be used. The abundant data already available for the perception by man of differences of brightness, such as that published by Nutting (32) in 1920, obtained with refined apparatus much of which can not be used for bees, would necessarily give man an unfair advantage in the comparison. In these experiments, therefore, human subjects were tested with the same apparatus as was used for bees.

The subjects were brought into the dark room one at a time and placed in such a position that they faced toward the two screens *f* and *g*. After allowing from one to two minutes for adaptation of the eyes to the darkness (corresponding to the time allowed for the bees to feed), the lamps in boxes *c* and *d* were turned on and the subject was asked which spot of light appeared the more brilliant to him. After he had answered, others (a total of from 1 to 11 in different tests) were brought in and tested similarly. Then the relative intensity of illumination by the two lamps was changed and the same or other subjects tested again. Seventy-five-watt lamps were used in all tests, set to illuminate the ground-glass screens at intensities in the ratios 1.0 : 1.0, 0.9 : 1.0 : 0.8 : 1.0, and 0.7 : 1.0. The positions of boxes *c* and *d* were interchanged frequently, and every known precaution was taken to avoid giving the subjects any clues as to which area really was the brighter. Most of the subjects did not even know how the apparatus was constructed until after the tests were completed.

#### RESULTS

The results for bees are presented in Table 7, and those for human subjects in Table 8. Figures 12 and 13 present graphically the results for bees, the former with illumination by 10-watt lamps, and the latter with illumination by 75-watt lamps. It will be seen that when the illuminations of the two areas were the same the number of responses to each, for both bees and human beings, was the same with the exception of the 30 initial responses recorded for bees when the 10-watt lamps were used. (Fig. 12.) That this exception is due to the small number of bees employed is indicated by the fact that the observation of 1,150 and 1,000 responses, respectively, in the other two experiments with intensity ratio of 1.0:1.0 gave results almost exactly 1.0:1.0. (Table 7.) When the intensity of illumination for the one area was lowered to 0.9 (Table 7, intensity ratio 0.9:1.0) the response of bees to the dimmer illumination also decreased in most experiments, but when the intensity was further lowered to 0.8, the response of bees to the dimmer in all experiments increased again until more than half were going to the dimmer area. (Table 7 and Figures 12 and 13.) This indicates that bees do not distinguish between illuminated areas under these conditions even when the intensity of one illumination is reduced to 80 per cent of the intensity of the other. When it was reduced to 0.7, however, in most experiments the dimmer area attracted fewer bees than the brighter, and when reduced to 0.6, 0.5, 0.4, and 0.3 it attracted on the whole fewer and fewer bees, although the decrease in response of the bees was not directly proportional to the decrease in intensity of the variable illumination.

TABLE 7.—Summary of experiments to test the ability of bees to distinguish between the intensity of illumination of two illuminated areas, with results for each ratio of intensities used

AREAS ILLUMINATED BY 10-WATT LAMPS \*

Variable illumination in terms of constant illumination	Bees used in observing total responses	Total responses observed	Total responses to variable illumination, in terms of total responses to constant illumination	Bees used in observing initial responses	Initial responses to variable illumination, in terms of initial responses to constant illumination
Ratio	Number	Number	Ratio	Number	Ratio
1.0	100	1,150	1.003	30	1.31
.9	30	150	.90	30	.67
.8	30	150	1.11	30	1.14
.7	30	200	1.06	30	.67
.6	30	150	.67	30	.88
.5	100	900	.77	40	.29
.4	30	150	.79	30	.76
.3	30	150	.61	30	.36

AREAS ILLUMINATED BY 75-WATT LAMPS \*

1.0	60	1,000	1.008		
.9	30	150	.92	30	1.14
.8	30	150	1.03	30	1.50
.7	30	150	.76	30	.67
.6	30	150	.60	30	.43
.5	30	150	.61	30	.50
.4	30	150	.52	30	.20
.3	30	150	.50	30	.67

\* For the 10-watt lamp at the distance of 40 cm., the minimum distance used, the intensity of illumination on the screen was approximately 45 meter candles; for a 75-watt lamp at the same distance, approximately 580 meter candles.

TABLE 8.—Summary of results of experiments to test the ability of human beings to distinguish between the intensities of illumination of two illuminated areas,<sup>a</sup> with results for each ratio of intensities used

Variable illumination in terms of constant illumination	Subjects used	Correct choices	Incorrect choices	Total choices	Correct choices in terms of total choices
Ratio	Number	Number	Number	Number	Per cent
1.0	2	2	0	2	100.0
.9	12	15	9	24	62.5
.8	9	11	2	13	84.6
.7	8	8	0	8	100.0

<sup>a</sup> Areas illuminated by 75-watt lamps. At the distance of 40 cm. the intensity of illumination was approximately 580 meter candles.

It will be observed in Figures 12 and 13 that for total responses the results are clearer and more consistent (the curves smoother) with the use of the 75-watt lamp than with the 10-watt lamp. For initial responses neither lamp seemed to have any advantage. In comparing total responses with initial responses, however, the total responses give much more consistent results.

It may be concluded, therefore, that under the conditions of intensity, type of apparatus, and adaptation to darkness used here bees

begin to distinguish between two illuminated areas when the difference between their intensities of illumination is increased to that represented by the ratio 0.7:1.0, but do not distinguish between them if the difference is equal to or less than that represented by the ratio 0.8:1.0.

When these results are compared with results obtained with human subjects the difference is striking. When the areas were made equal in brightness the human subjects used could see no difference between them (Table 8), as was to be expected. When the brightness of one area was reduced to 0.9 of that of the other, however, 15 out of 24 subjects, or 62.5 per cent, selected it as the dimmer. When further reduced to 0.8, in 11 out of 13 tests, or 84.6 per cent, that area was

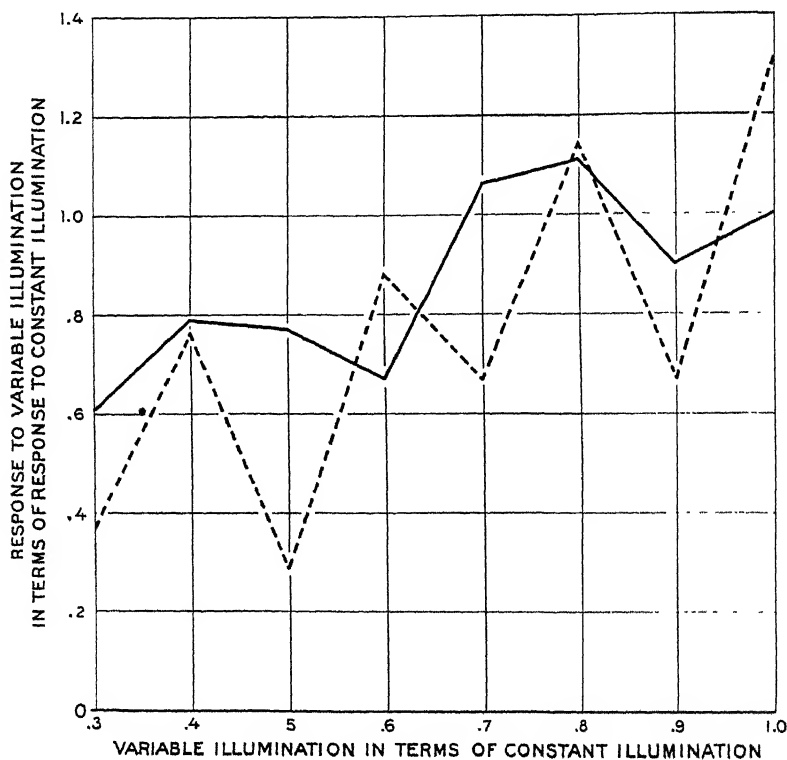


FIGURE 12.—Relative response of bees to illuminations of various relative intensities, when 10-watt lamps were used as sources of illumination. Broken line, averages of initial responses of bees, solid line, averages of total responses

selected as the dimmer, and when reduced to 0.7, all of the eight subjects selected it as the dimmer.

It may be concluded, therefore, that under the conditions of intensity, type of apparatus, and adaptation to darkness prevailing here, human beings can usually distinguish between two illuminated areas when the difference is only that represented by the ratio 0.9:1.0 and can unmistakably distinguish between them when the difference is as much as that represented by the ratio 0.7:1.0; and, accordingly, that human beings are considerably more sensitive than bees to differences in intensity of illumination.

Such a qualitative statement as the above seems amply justified by these experiments, but to state quantitatively how much more sensitive men are than bees requires a great deal of caution, as indeed does the quantitative comparison of the behavior of man with that of any other animal. Caution is necessary here first of all because of a difference in the nature of the response; the human subjects were asked to state which of the two areas appeared brighter (or dimmer) to them, whereas one obtained similar information from the bees by observing their movements. With human subjects the response is simple,

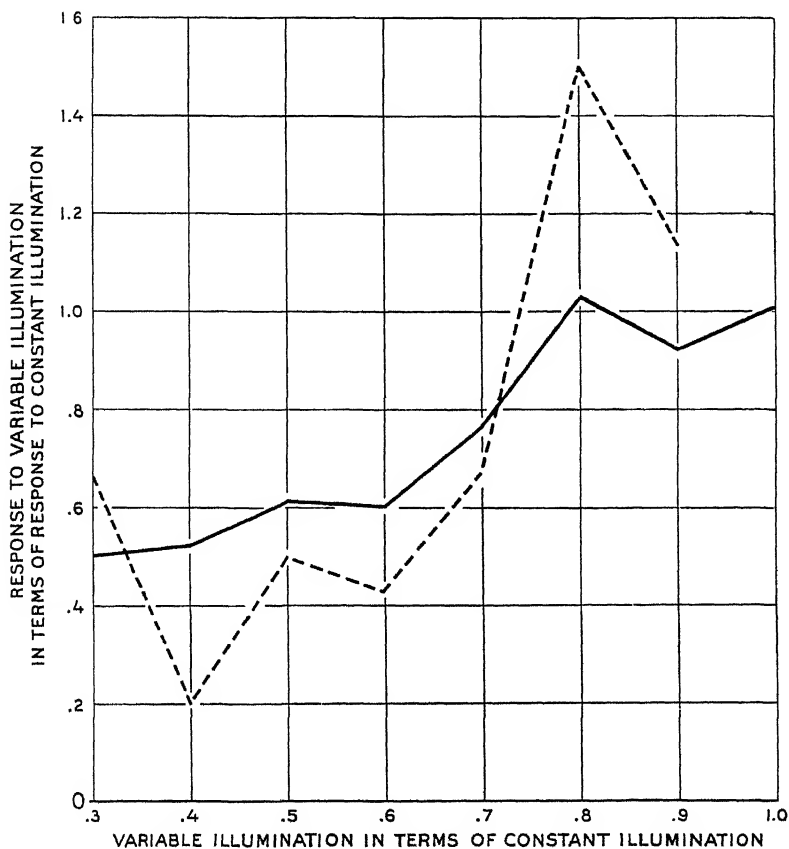


FIGURE 13.—Relative response of bees to illuminations of various relative intensities, when 75-watt lamps were used as sources of illumination. Broken line, averages of initial responses of bees; solid line, averages of total responses

involving only sight and speech in a reaction hardly more than a simple reflex. With bees the response may be enormously complex, since the reaction is in all probability an attempt to escape from the box, which is influenced on occasion by a difference in the intensities of two separate illuminations, and is consequently a very complicated reflex, conditioned not only by the metabolic condition of the bee but by all its past experiences in dealing with such situations.

Caution is necessary, secondly, because of a fact which may be closely correlated with the first; namely, a wide variation in the re-



actions of the bees under identical conditions. One of the most striking examples of this was a test in which reactions were obtained of 10 bees to two intensities whose ratio was 0.3:1.0 (with 10-watt lamps); of the first 50 reactions obtained 24 were toward the intensity of 1.0, while 26 were toward the intensity of 0.3, whereas of the last 50 reactions obtained with 10 other bees, 38 were toward the intensity of 1.0 and 12 toward the intensity of 0.3. If one had stopped with the first 50 reactions it would have appeared that the bees did not distinguish between intensities so widely different as 1.0 and 0.3. The detailed record of these two tests is given in Table 6. Until one can control more factors influencing the reactions of bees in such experiments as these, one must simply use such large numbers that variations will tend to fall equally on each side of the correct value.

Although a definite quantitative comparison of acuity in bees and in human beings in distinguishing differences of brightness is thus not permissible from the results of these experiments, it is clear that human beings are considerably more acute in this respect than bees, and the method here followed offers the possibility of comparing other insects with bees, provided large numbers of each insect are used.

#### CONCLUSION

From the results of these experiments it appears that neither the conclusion of Von Frisch and Kühn that bees distinguish only enormous differences in brightness, nor that of Von Hess that bees are practically as acute as man in this ability is correct. The more nearly correct conclusion seems to be in the nature of a compromise between the two, namely, that bees begin to distinguish between two illuminated areas when the intensity of one is reduced to at least 70 per cent of the intensity of the other, whereas human beings distinguish between them equally well when the intensity of the one is reduced to only 90 per cent of that of the other.

#### GENERAL CONCLUSIONS

The upper limit of the spectrum for the honeybee extends to at least wave length  $677\text{ m}\mu$  in the red. The difference between this result and that of  $650\text{ m}\mu$  found by Kühn is probably due to a difference in the intensity of the spectral light used in the two sets of experiments. The lower limit was not ascertained in the research here reported, but according to Kühn it extends to at least  $313\text{ m}\mu$ .

The point of maximum stimulative efficiency for the honeybee is in the yellow-green at about  $553\text{ m}\mu$ , which corresponds rather closely with that for man. From this point the efficiency decreases more rapidly for bees than for man toward the longer wave lengths, but more slowly than for man toward the shorter wave lengths, being at  $431\text{ m}\mu$  still fully 10 per cent of the maximum.

Untrained bees, when allowed to walk toward two sources of light of the same quality, placed near together at the end of a rectangular box, go to the brighter more often than to the fainter, but not in direct proportion to the relative brightness of the two. The curve representing the relation between relative brightness and relative magnitude of response is a polynomial one, of such form that the curve showing the relation between the logarithms of the two related variables is an approximately straight line.

The presence of chroma vision in bees, already strongly indicated by the work of previous investigators, is more clearly established.

For the moderate intensities used in these experiments (Part III), the bee begins to distinguish between two illuminated areas when the illumination of one is reduced to an intensity 70 per cent as great as that of the other. The ability to distinguish is slightly less pronounced when the intensity of illumination is low (45 meter candles on the brighter area) than when it is high (580 meter candles on the brighter area).

Human beings, tested with the same apparatus as that used for bees (using the higher intensity of illumination), begin to distinguish between the brightness of two illuminated areas when the intensity of one is reduced to 90 per cent of that of the other; this distinction is fairly accurate when the intensity of the one is reduced to 80 per cent, and is quite accurate when the reduction reaches 70 per cent. Human beings are, therefore, considerably more acute than bees in ability to distinguish differences in brightness.

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# THE VALUE OF ALFALFA AS A SOURCE OF VITAMIN A IN SORGHUM-GRAIN RATIONS<sup>1</sup>

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## INTRODUCTION

In previous papers (8, 9)<sup>2</sup> from this laboratory it was shown that the sorghum grains hegari and yellow milo are strikingly deficient in vitamin A. A quantitative measure of the vitamin A content of these grains and of yellow corn showed that hegari was only one-twentieth as rich in vitamin A as yellow corn. The small amount of vitamin A in hegari was proved to be insufficient for continued growth at a normal rate of young albino rats. All the animals dependent upon hegari for vitamin A exhibited a low degree of health and vigor, with marked susceptibility to infections of various kinds and a striking failure in reproduction and rearing of their young.

As the sorghum grains are drought-resistant and high-yielding, with a minimum amount of water, they are widely used in animal-feeding rations in place of yellow corn, which can not be successfully grown in the arid Southwest. For this reason it seemed advisable to investigate the supplementary value of alfalfa, a crop extensively grown in the Southwest and known to be rich in vitamin A, as a vitamin A supplement to hegari-grain rations.

## EXPERIMENTAL METHODS AND MATERIALS

### PLAN OF PROCEDURE

Albino rats were taken at the time of weaning from the stock colony reared on Sherman's diet B, composed of two-thirds ground whole wheat, one-third whole-milk powder, and salt equal to 2 per cent of the weight of the wheat, and they were placed upon experimental rations composed largely of hegari supplemented with alfalfa-leaf meal as an additional source of vitamin A and made adequate in other dietary essentials as indicated in preliminary experiments by the addition of casein, yeast, and mineral salts. The composition of these experimental diets is given in Table 1.

TABLE 1.—Percentage composition of the experimental rations

Ration No.	Hegari	Corn	Alfalfa- leaf meal	Casein	Yeast	Calcium lactate	Sodium chloride
3.....	0	73	0	9	<sup>a</sup> 15	2	1
37.....	73	0	0	9	15	2	1
38.....	72	0	1	9	15	2	1
39.....	68	0	5	9	15	2	1
40.....	63	0	10	9	15	2	1
41.....	58	0	15	9	15	2	1
42.....	48	0	25	9	15	2	1
4.....	71	0	0	9	15	2	<sup>b</sup> 1

<sup>a</sup> The 2 per cent yeast used in the early work was found to be inadequate for continued successful reproduction and lactation.

<sup>b</sup> 2 per cent cod-liver oil added.

<sup>1</sup> Received for publication Oct. 13, 1930; issued April, 1931.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 431.

The whole hegari grain was obtained from the University of Arizona experiment farm and ground to a flour. Whole yellow corn was secured from Kansas and likewise finely ground.

High-grade alfalfa-leaf meal containing 20 to 22 per cent protein was obtained from a local milling company. The casein was a commercial unextracted product. The dried, powdered yeast used was obtained from the Northwestern Yeast Co.

Vitamin D was provided by the incorporation of 0.5 gm. of cholesterol from 1,000 gms. of food which had been irradiated for 30 minutes at a distance of 2 feet from an ultra-violet lamp of the Hanovia mercury-vapor quartz type.

Litter mates, matched as to size and sex as far as possible, were placed upon the different experimental rations. The living conditions of all the animals were identical, the rats being reared in square metal cages with false screen bottoms which were changed daily. Not more than six animals were kept in one cage. The experimental rations and distilled water were fed *ad libitum*. Weekly records of the weights of all the animals were kept throughout the duration of the experiment, and for a limited time the food intakes were measured and compared.

All of the rations were palatable to the animals, although a greater tendency to scatter the rations containing the higher percentages of alfalfa-leaf meal was noted. The food intake per gram of gain in body weight increased somewhat as the percentage of alfalfa-leaf meal in the ration was increased because of the lower caloric value of these rations.

Males and females were housed together at all times, and the age of animals at mating and the frequency of their matings were not controlled. Pregnant females were separated from the lot cages at least one week before parturition and were weighed daily until after the young were delivered. The nesting material provided the mothers after their young were born consisted of finely cut pure cellulose. The size of the litter was not restricted, each mother being allowed to suckle all her young.

The young animals were weighed as a litter weekly until they were 4 weeks of age, at which time they were weighed separately, numbered, and recorded as individuals, and the mother was returned to the lot from which she came.

Litter mates, matched as to size and sex as far as possible, were placed upon the different experimental rations. Three males and three females were placed upon each ration at the beginning of the experiment, and more animals were started upon these same diets, with the exception of diet 38, from time to time as they became available. A greater number of animals were placed upon ration No. 37 because this ration early showed signs of inadequacy, and further evidence of this kind seemed desirable. In every case a representative pair of young rats from the first litter of each of the three original females, and as far as possible from all females on each ration, was continued on the diet of the parent animals. In this way a study of the effect of the ration on four generations was made.

Criteria for judging the adequacy of each experimental ration consisted in such factors as have been emphasized by Sherman (4) to be related to optimum health, namely, rapidity of growth, maximum size obtained, age of maturity, success of reproduction and rearing of

the young, and physical vigor of parents and offspring, including the duration of their prime of life.

That vitamin A can be stored in the animal body and that the amount stored depends upon the concentration of vitamin A in the ration upon which the animal feeds has been proved conclusively by Sherman and his coworkers (3, 5, 7). Thus, young animals may have at weaning a considerable store of vitamin A, the amount depending upon the concentration of vitamin A in the mother's diet. Such a store is a nutritional asset of great importance in building up reserve resistance to infection, etc. Within limits, then, a ration to be considered adequate should provide opportunity for storage of this vitamin.

Further to check the adequacy of vitamin A in each of the experimental diets, the opportunity which each ration provided for storage of vitamin A was measured by determining the survival period of young rats, taken at the time of weaning, from mothers reared upon the hegari-alfalfa rations and placed on a vitamin-A-free ration composed of extracted casein 18 per cent, Osborne and Mendel's salt mixture 4 per cent, dried brewer's yeast 10 per cent, cornstarch 67 per cent, and sodium chloride 1 per cent, with 0.5 gram irradiated cholesterol added to each 1,000 grams of the diet. A representative pair of young rats taken from each litter was used in this way. Health observations were made, and a record was kept as to the time of appearance of symptoms of vitamin A deficiency, such as cessation of growth, ophthalmia, etc., which indicated exhaustion of body stores of this vitamin. All the animals were autopsied, and the survival periods of those rare animals showing neither premortem nor post-mortem indications of vitamin A lack were not included in the results as tabulated.

## EXPERIMENTAL DATA

### GROWTH RECORDS

In Table 2 appear the growth records of male rats reared upon the experimental hegari-grain rations containing different percentages of alfalfa-leaf meal as a supplementary source of vitamin A (rations 38, 39, 40, 41, and 42). These are compared with the rates of gain for a similar period of time of male rats dependent upon hegari (ration 37) or yellow corn (ration 3) for all of their vitamin A and also with the growth performances of males receiving a liberal additional supply of vitamin A in the form of 2 per cent cod-liver oil (ration 4). The tabulated results are shown graphically in Figure 1.

TABLE 2.—*Growth records of male rats on all experimental diets*

### FIRST GENERATION

No. and composition of ration	Rats	Average weight at weaning	Average weight at 16 weeks	Average weight at 24 weeks
	Number	Grams	Grams	Grams
3, yellow corn, no alfalfa.....	6	59	288	343
37, hegari, no alfalfa.....	12	57	263	305
38, hegari and 1 per cent alfalfa.....	3	51	276	327
39, hegari and 5 per cent alfalfa.....	7	56	300	350
40, hegari and 10 per cent alfalfa.....	6	59	304	357
41, hegari and 15 per cent alfalfa.....	5	58	304	363
42, hegari and 25 per cent alfalfa.....	6	53	283	329
4, hegari and 2 per cent cod-liver oil.....	5	56	325	368



TABLE 2.—Growth records of male rats on all experimental diets—Continued  
SECOND GENERATION

No and composition of ration	Rats	Average weight at weaning	Average weight at 16 weeks	Average weight at 24 weeks
		Number	Grams	Grams
3, yellow corn, no alfalfa.....	5	53	260	295
37, hegari, no alfalfa.....	12	35	219	256
38,* hegari and 1 per cent alfalfa.....	3	51	282	---
39, hegari and 5 per cent alfalfa.....	5	59	294	358
40, hegari and 10 per cent alfalfa.....	5	57	310	369
41, hegari and 15 per cent alfalfa.....	3	53	290	347
42, hegari and 25 per cent alfalfa.....	6	48	282	339
4, hegari and 2 per cent cod-liver oil.....	4	57	334	380

## THIRD GENERATION

3, yellow corn, no alfalfa.....	3	55	256	286
37, hegari, no alfalfa.....	4	42	198	237
39, hegari and 5 per cent alfalfa.....	4	49	290	342
40, hegari and 10 per cent alfalfa.....	5	52	296	356
41, hegari and 15 per cent alfalfa.....	3	63	294	363
42, hegari and 25 per cent alfalfa.....	6	54	304	373
4, hegari and 2 per cent cod-liver oil.....	4	58	291	360

## FOURTH GENERATION

3, yellow corn, no alfalfa.....	3	55	259	286
37, hegari, no alfalfa.....	1	51	218	234
39, hegari and 5 per cent alfalfa.....	3	57	300	361
40, hegari and 10 per cent alfalfa.....	3	51	286	342
41, hegari and 15 per cent alfalfa.....	3	47	280	343
42, hegari and 25 per cent alfalfa.....	4	48	200	334
4, hegari and 2 per cent cod-liver oil.....	3	53	308	362

\* Ration No. 38 was discontinued in second generation.

Table 2 shows conclusively, as has been previously noted (8), that hegari as the sole source of vitamin A in a ration believed to be other-

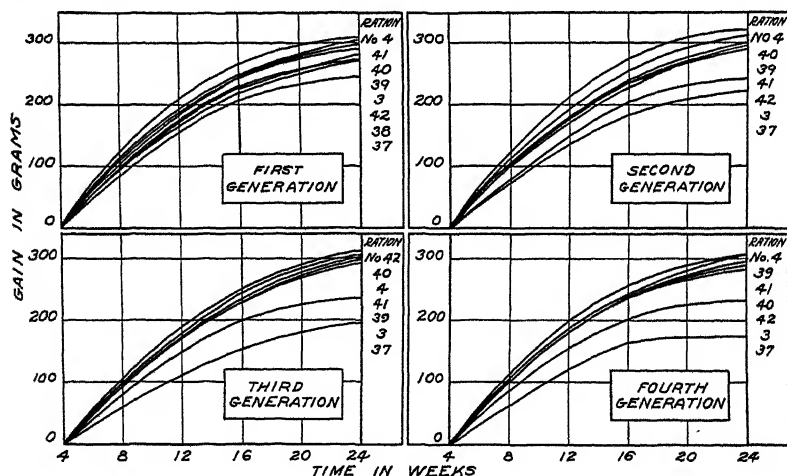


FIGURE 1.—Average growth records of male rats reared on hegari-grain rations containing varying percentage of alfalfa-leaf meal as a supplementary source of vitamin A. Five per cent of alfalfa-leaf meal (ration No. 39) apparently provides sufficient vitamin A to support a normal rate of growth. Increasing the amount of alfalfa meal to 10, 15, and 25 per cent (rations No. 40, 41, and 42, respectively) resulted in no significant differences in growth rate

wise adequate does not provide for an optimum rate of growth of male rats. This inability of hegari to supply sufficient vitamin A for young, growing animals becomes more apparent in each succeeding

generation, as evidenced by a growth rate progressively more retarded in the later generations.

Enriching the vitamin A value of the ration by the incorporation of alfalfa-leaf meal largely compensates for this deficiency of the grain sorghum itself and makes possible a rate of gain more closely approximating the optimum. Whereas 1 per cent of alfalfa-leaf meal resulted in a marked stimulus to growth, the 5 per cent level of feeding gave superior results. Further increases of the vitamin A content of the ration by the use of greater percentages of alfalfa-leaf meal resulted in no greater growth, no significant differences in the growth of animals receiving 5, 10, or 15 per cent of alfalfa meal being observed. In other words the greatest growth rates which were obtained by the use of alfalfa-leaf meal as a supplement to the sorghum grain, hegari, therefore, resulted upon the inclusion of 5 per cent of this material rich in vitamin A. The slightly inferior rate of growth of the males receiving the highest percentages of alfalfa meal was believed to be due to the extensive amount of roughage furnished by the alfalfa. Cases of diarrhea among the animals receiving 15 and 25 per cent of alfalfa meal were common. Just what effect the relatively high fiber content of the alfalfa had upon the assimilation of vitamin A can not be stated.

The somewhat greater weight in most cases of the males reared upon the hegari ration supplemented with cod liver oil as well as the poorer results obtained in the later generations with the yellow corn ration suggest a possible dietary deficiency other than vitamin A. Inadequate vitamin D in a grain ration not sufficiently fortified with calcium is suspected, such a deficiency being remedied by the vitamin D in cod-liver oil, or the calcium and vitamin D in alfalfa, but remaining as a possible limiting factor of the unsupplemented hegari and yellow-corn rations. That vitamin A is the first limiting factor in hegari ration No. 37, which is remedied by the addition of alfalfa or cod-liver oil, is made evident by the data presented later in this paper relative to the comparative stores of vitamin A in the bodies of young rats reared on the hegari and the alfalfa-supplemented hegari rations.

#### REPRODUCTION RECORDS

Evans and Bishop (2) have shown that faulty diet resulting in undernutrition may prevent or postpone the attainment of sexual maturity and greatly affect the ovulation rhythms of female animals reared upon inadequate rations. Observations of the age of rupture of the vaginal membrane of females reared on the experimental hegari and hegari-alfalfa meal rations showed no consistent differences in the age of attainment of sexual maturity of these females that could be correlated with their diet, and no definite abnormality of cycle was noted. The vaginal membrane ruptured in the majority of cases about the fifty-fifth day even in females reared upon ration No. 37, which was low in vitamin A.

Daily microscopic examination of the vaginal smears of many of the females over a limited period of four months, however, showed a strikingly definite abnormality of the ovulation rhythm of the females dependent upon hegari as their only source of vitamin A. A disturbance, which has been pointed out by Evans and Bishop (2) as highly characteristic of lack of vitamin A, was observed in 100 per cent of the females raised on ration No. 37, namely, a prolongation of the oestral

desquamative change in the vaginal epithelium. For the entire period of observation in most of the females, the vaginal smear consisted chiefly of the cornified cells, which normally characterize the actual period of ovulation only. Both epithelial cells and leucocytes appeared intermittently, but cornified cells were always present in abundance. Ovulation took place, although irregularly, and a few litters were born to these females.

No similar abnormality of oestrus was observed in the same number of females reared on the grain rations in which alfalfa-leaf meal supplemented the vitamin A in hegari by furnishing additional vitamin A.

Sherman and MacLeod (6) have shown that—

A proportion of vitamin A in the food sufficient to support normal growth may still be insufficient for the added nutritive demand of successful reproduction and lactation.

Reproduction records for the first year of life (approximately one-third of the normal life span) of females reared upon the different hegari-alfalfa experimental rations appear in Table 3.

TABLE 3.—*First-year reproduction records of first-generation females reared upon ration Nos. 37, 38, 39, 40, 41, 42, 3, and 4*

Ration No.	Females used	Average litters per female	Average size of litters	Average young per female	Average young weaned per female		Average weight in grams of young at weaning (28 days)	
	Number	Number	Number	Number	Number	Per cent	♂	♀
37-----	12	2.8	5.2	14.6	4.8	32.8	39.0	35.8
38-----	3	5	6.0	30.0	21.9	73.0	49.8	47.3
39-----	6	5	7.0	35.0	31.3	89.4	61.9	55.5
40-----	6	5	7.5	37.5	34.2	91.2	61.1	56.5
41-----	5	5	7.5	37.5	34.0	90.7	56.5	54.0
42-----	6	5	7.0	35.0	31.5	90.0	52.4	51.0
3-----	6	5	7.1	35.5	31.6	89.0	54.0	51.4
4-----	5	5	8.0	40.0	36.3	90.75	58.5	56.0

The supplemental value of alfalfa as an additional source of vitamin A in hegari-grain rations is again evident from the comparative female reproduction records appearing in Table 3.

In the first year of life the average female reared on ration No. 37, in which hegari furnishes all the vitamin A, produced approximately three litters with an average size of 5.2 young but was successful in rearing but 32.8 per cent of her offspring. At weaning time (28 days of age) they were undersized and weaklings with an average weight of 39 gm. for the males and 35.8 gm. for the females. Addition of 1 per cent alfalfa-leaf meal improved the reproductive performance of the females considerably, enabling them to bear larger litters more frequently and to suckle to the weaning age a larger percentage (73 per cent) of their young. However, the results were far from optimum at this low vitamin level (1 per cent of alfalfa-leaf meal). With the inclusion of 5 per cent of alfalfa-leaf meal in the hegari ration, a much more nearly optimum reproductive performance was obtained. Not only did the females produce more young and wean a larger percentage of them, but the young themselves were in better physical condition, as indicated by their general appearance and liveliness and their decidedly greater weaning weight.

Increasing the vitamin A value of the ration still further by the use of greater percentages of alfalfa-leaf meal (10, 15, and 25 per cent) did not materially improve the reproduction records of the females

during the first year. However, the period of reproductive life of the females on ration No. 39 is shorter than that of females which received the higher percentages of alfalfa. For example, female No. 496 reared on ration No. 40 continued to be fertile for 196 days after her sister, receiving only 5 per cent of alfalfa-leaf meal, had ceased to bear young.

Figure 2 shows graphically the comparative growth and reproduction performances of representative litter mates reared upon ration No. 37, low in vitamin A, and ration No. 40, in which the inadequate vitamin A value of the hegari is plainly compensated for by the addition of 10 per cent of alfalfa-leaf meal.

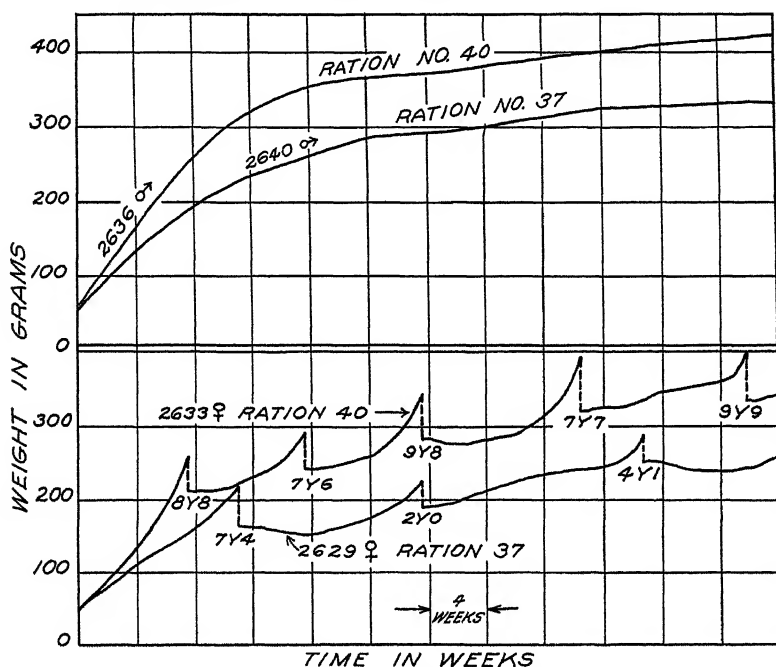


FIGURE 2.—Comparative growth and reproduction records of litter mates reared on hegari-grain rations with and without a supplementary source of vitamin A. Female 2629, reared on ration No 37, bore 13 young in the first year and raised but 5 (38.7 per cent). She died five weeks later with undelivered young. On the other hand, her litter sister 2633, who had received 10 per cent of alfalfa-leaf meal (ration No. 40), bore 40 young in the same length of time and successfully suckled 95 per cent of them. The break in the female growth curves indicates loss of weight upon delivery of young. The number before the Y refers to the number of young born and that after the Y indicates number of young weaned.

#### STORAGE OF VITAMIN A

The relative stores of vitamin A in the 28-day-old young of females reared upon the sorghum grain alfalfa rations as measured by determination of their survival time when transferred to a ration free from all vitamin A are presented in Table 4 and shown graphically in Figure 3.

That the young which do live to be weaned on ration No. 37 start out in life with but a very small reserve supply of vitamin A and are therefore poorly equipped to withstand infection is shown by the fact that within a week ophthalmia appears, a maximum gain of only 11 gm. is reached, and the animals survive on the vitamin-A-free

ration only 22.6 days. On the other hand, the young of females that have been reared on rations Nos. 38, 39, 40, 41, and 42, containing 1, 5, 10, 15, and 25 per cent, respectively, of alfalfa-leaf meal as a supplementary source of vitamin A possess greater bodily stores of this vitamin at weaning and are better able to withstand subsequent deprivation. It is quite apparent, too, that the increasing percentage

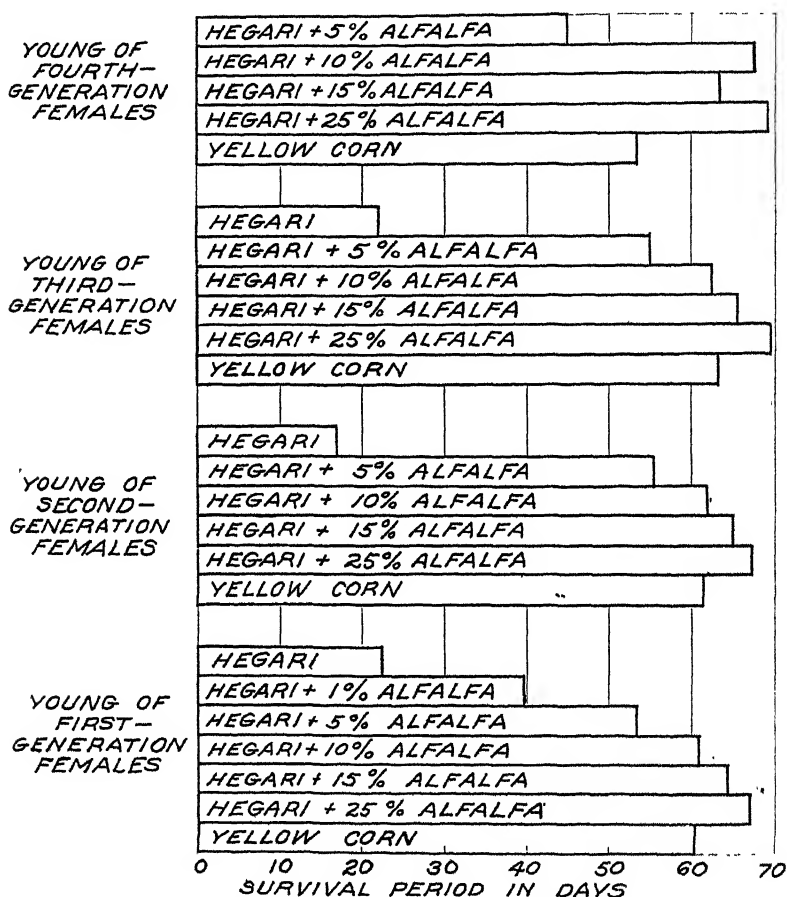


FIGURE 3.—Increase of stored vitamin A in rats as measured by the survival period upon a vitamin-A-free diet, when increasing percentages of alfalfa are fed in the ration. The increment of increase becomes progressively smaller with each increasing percentage of alfalfa leaf meal fed.

of alfalfa-leaf meal in the ration is reflected directly in the increasingly greater bodily store of vitamin A in the young at weaning for a prolongation of the survival period (from 22.6 to 67 days), a greater delay in the appearance of the signs of infection which characterize the lack of vitamin A, and the attainment of a larger maximum weight before decline set in, accompanied the inclusion in the ration of increasing percentages of alfalfa.

TABLE 4.—Storage of vitamin A in 28-day young of females reared on experimental rations as shown by their survival on vitamin-A-free diet

## YOUNG OF FIRST-GENERATION FEMALES

Ration No. and composition	Mothers represented	28-day-old offspring placed on vitamin-A-free diet	Average survival on vitamin-A-free diet
	Number	Number	Days
37, hegari, no alfalfa.....	12	43	22.6
38, hegari and 1 per cent alfalfa.....	3	16	39.6
39, hegari and 5 per cent alfalfa.....	6	36	53.5
40, hegari and 10 per cent alfalfa.....	6	40	61.0
41, hegari and 15 per cent alfalfa.....	5	32	64.5
42, hegari and 25 per cent alfalfa.....	6	40	67.0
3, yellow corn.....	6	42	60.3

## YOUNG OF SECOND-GENERATION FEMALES

37, hegari, no alfalfa.....	<sup>a</sup> 2	4	17.0
39, hegari and 5 per cent alfalfa.....	4	22	55.4
40, hegari and 10 per cent alfalfa.....	6	22	62.0
41, hegari and 15 per cent alfalfa.....	5	18	65.0
42, hegari and 25 per cent alfalfa.....	6	25	67.3
3, yellow corn.....	4	20	61.8

## YOUNG OF THIRD-GENERATION FEMALES

37, hegari, no alfalfa.....	2	2	22.0
39, hegari and 5 per cent alfalfa.....	3	14	55.0
40, hegari and 10 per cent alfalfa.....	4	24	62.6
41, hegari and 15 per cent alfalfa.....	3	20	65.8
42, hegari and 25 per cent alfalfa.....	4	22	69.7
3, yellow corn.....	4	16	63.1

## YOUNG OF FOURTH-GENERATION FEMALES

37, hegari, no alfalfa.....	( <sup>b</sup> )	-----	-----
39, hegari and 5 per cent alfalfa.....	3	12	45.0
40, hegari and 10 per cent alfalfa.....	4	14	67.6
41, hegari and 15 per cent alfalfa.....	3	12	63.4
42, hegari and 25 per cent alfalfa.....	4	18	69.2
3, yellow corn.....	4	16	53.5

<sup>a</sup> Only 2 out of 9 second-generation females on ration No. 37 raised young.<sup>b</sup> No young raised by the 1 fourth-generation female on ration No. 37.

However, it may be noted here, as has been previously shown by Sherman and his coworkers (3, 6), that the store of vitamin A does not increase in arithmetical proportion to the concentration of this vitamin in the ration. (Fig. 3.) The animals appear to use the vitamin less economically as its concentration in the food is increased. Thus enriching the vitamin A value of the rations with 1 per cent of the supplement, alfalfa-leaf meal has made possible an increase in storage of 77 per cent over that in animals which have been dependent upon hegari for all their vitamin A (ration No. 37). An increase in storage of vitamin A of approximately 137, 170, 185, and 196 per cent was found in rations containing 5, 10, 15, and 25 per cent, respectively, of alfalfa-leaf meal. It may be seen that the increment of increase in the store becomes smaller as the concentration of vitamin A in the rations is increased by the use of the greater percentages of alfalfa.

Whereas 1 per cent of alfalfa increased the store of vitamin A in the bodies of the young rats by 75.2 per cent over that in the animals on ration No. 37, the addition of 4 per cent more resulted in a further increase of 35.1 per cent, the next 5 per cent (ration No. 40) increased the store 14 per cent more, whereas the next additional 5 per cent gave a further increase of but 5.7 per cent, and the final increase of 10 per cent of alfalfa increased the amount of the reserve supply by only 3.8 per cent, which shows a decrease in efficiency of storage of the vitamin as the basal ration was enriched.

#### LONGEVITY RECORDS

A definite tendency toward longer life as the diet is made richer in vitamin A has been demonstrated by Sherman and Campbell (4) and again by Batchelder (1). Although longevity records of the animals on all of the experimental hegari-alfalfa rations are incomplete, the data at hand give further proof of the inadequacy of hegari as a source of vitamin A and of the supplemental value of alfalfa-leaf meal.

At the time of the present writing more than two years (849 days) have elapsed since the first-generation animals were transferred from the stock colony to the experimental rations. In this period only one animal on a hegari ration supplemented with alfalfa has died and this was a female who developed a large tumor weighing 500 gm. after having shown an excellent reproduction record.

On the other hand, of the 22 first-generation animals reared upon ration No. 37 in which hegari serves as the only source of vitamin A, with the exception of one female who has never raised a litter and is still living, all of the animals have died after a relatively short life span (552 days average for the males and 321 days for the females). Most of the females on this ration were pregnant at the time of death, dying during parturition. Autopsy records revealed undeveloped young. The second-generation animals being less fortified with vitamin A at birth, and therefore less thrifty than their parent animals taken from the stock colony, exhibited a shorter life span (287 days average for the males and 213 days for the females). Only six third-generation, and two fourth-generation rats were raised on this ration, the families therefore practically dying out in the third generation.

Although all the animals, save one, on the rations containing alfalfa-leaf meal are still living, a great difference in vigor and in their outward appearance may be noted. Animals on ration No. 39 containing 5 per cent of the alfalfa-leaf meal show signs of old age, thin unkempt hair, lack of vitality, and are losing weight, whereas the animals of the same age which have received 10 or 15 per cent of alfalfa are in much better physical condition, and better appearing in every way. It would seem that 5 per cent of alfalfa-leaf meal enabled the young males to grow at a normal rate and the females to reproduce successfully for a time at least, but the drain upon the mother animal is excessive, the reproductive period is shorter, and the animals pass their prime of life at a relatively earlier age and in all probability will show a shorter life span.

## SUMMARY AND CONCLUSIONS

In order to determine the supplementary value of alfalfa-leaf meal as a food material rich in vitamin A for use in sorghum-grain rations, albino rats of the same nutritional history, matched as to sex, weight, and litter, were placed upon hegari (a sorghum grain) rations containing alfalfa-leaf meal ranging in amount from 1 to 25 per cent (ration Nos. 38 to 42, inclusive). The amount of alfalfa-leaf meal which should be incorporated into the ration to compensate for the lack of vitamin A in the hegari itself was indicated by such criteria as the ability of the ration to support an optimum rate of growth in young male animals, to permit females to reproduce and successfully suckle many vigorous offspring, to provide opportunity for storage of sufficient vitamin A to increase the body ability to resist infection, and to enable the animals to live to a healthy old age.

Growth records show that 5 per cent of alfalfa-leaf meal provides sufficient vitamin A to promote good growth. No better growth was obtained with the higher percentages of alfalfa leaf meal.

Reproduction records show that females receiving 5 per cent of alfalfa-leaf meal produce healthy, thrifty young and are successful in suckling a large percentage of them. However, 10 per cent of alfalfa-leaf meal insures a longer reproductive life, the females on ration No. 40 raising many young after the reproductive life of females on ration No. 39 is passed.

A measure of the relative stores of vitamin A in the bodies of the young at weaning time shows an increasing store as the percentage of alfalfa-leaf meal is increased. The increment of increase in store with each percentage increase in the ration of the vitamin-A-rich alfalfa becomes decidedly less when more than 10 per cent is incorporated in the ration.

Longevity records, although incomplete, show that whereas 5 per cent of alfalfa-leaf meal results in striking prolongation of life, when 10 per cent is incorporated in the ration the animals show signs of old age at a still later date and will in all probability live longer.

Taking all points into consideration, it would appear that whereas incorporation of 5 per cent of alfalfa-leaf meal in the sorghum-grain ration largely compensates for the lack of vitamin A in the hegari itself resulting in a striking stimulus to growth, improving markedly the female reproductive performances, etc., the use of 10 per cent of alfalfa-leaf meal produces more nearly optimum results.

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## URINARY CALCULI IN SHEEP<sup>1</sup>

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### INTRODUCTION

The occurrence of calculi in sheep may not be more frequent than in other animals, but present knowledge of the subject indicates that it probably is. Possibly this trouble occurs much more often than is generally supposed, for if the calculi are small and few in number they may not noticeably affect the animal and therefore may escape detection. Furthermore, it is highly probable that a good many cases are missed because the average sheepman and some veterinarians do not recognize the symptoms.

Frequent references in early livestock literature to "gravel" and "stones," the terms most commonly used by sheepmen to designate calculi, indicate that they have long been known and recognized by experienced sheepmen. The cause of their formation has been attributed to (1) roots, particularly mangels; (2) hard water; (3) high condition; (4) lack of exercise; and (5) chilling from east winds in the early spring after shearing. When sheep are affected with calculi, the voiding of urine is restricted and, in some cases, is entirely prevented. Concerning this symptom, Youatt (14, p. 588-589)<sup>3</sup> says:

It is mostly seen in males, and particularly rams, while being got up for exhibition purposes, under the influence of rich highly stimulating food.

The chief cause of this defect as seen in sheep is due to the presence of minute sandy particles, which block up the urinary passage at the extreme end of the penis (worm). This gravelly matter is deposited from the urine while in the bladder, and when passing along the canal becomes arrested in the small worm-like appendix of the organ, thus shutting in the urine and preventing its escape. The deposition of sandy matter in the bladder is said by practical flock-masters to arise from the excessive use of mangels and other foods rich in sugar. We think, however, that the want of exercise and the habit of going for long periods without emptying the bladder, as do fat lazy sheep, has much to do with it.

A survey of the literature indicates that the calculi are usually produced by formations of calcium carbonate, calcium and aluminum phosphates, aluminum silicate together with kidney tissue urates, epithelium, etc. Michael (10) studied calculi of sheep for five years. He obtained data from lots of rams fed (1) grain and clover hay; (2) grain, hay, and corn silage; (3) grain, hay, and mangels; and (4) grain, hay, and sugar beets. In lots 1 and 2 no calculi were found, and the urinary organs were normal, but in lots 3 and 4 there were a number of cases of calculi, and all but four of the rams in lot 4 had distended bladders. Six of the 11 in lot 4 had enlarged gall bladders, 4 had enlarged hearts, 6 had enlarged kidneys, and most of them showed irritation in one or more parts of the urinary tract. Michael

<sup>1</sup> Received for publication Aug. 8, 1930; issued April, 1931.

<sup>2</sup> The writers are indebted to Samuel Breese, shepherd of the Purdue flocks who fed the experimental rations and assisted in obtaining the urine samples and in diagnosing the calculi cases; to Dr. J. R. Mohler, Dr. G. F. Creech, D. A. Spencer, of the United States Department of Agriculture, and Prof. Claud Harper for obtaining certain data on the lambs used in the experiments, to Elizabeth Heiss for the drawing shown as Figure 1; and to Dr. E. J. Kohl for making the photographs.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 446.

attributes the enlargement of the organs to the large amount of water ingested, which caused distention and increased the activity of the kidneys. These results would appear to indicate that the mangels and sugar beets were responsible for the formation of calculi.

Hager and Magath (4, 5, 6) produced vesical calculi experimentally in rabbits and guinea pigs. They catheterized the bladder and then introduced 0.5 c. c. of a 0.1 per cent alcoholic solution of salicylic acid, which produced a marked excoriation of the mucosa. After this injection, 1 c. c. of a 24-hour broth culture of *Proteus ammoniae* was introduced into the bladder through a catheter. This resulted in the production of a severe grade of incrustated cystitis which lasted for an indefinite period, the urine becoming markedly alkaline. These investigators recognized bacteria from two sources which they believe are concerned in "stone" formation; (1) endogenous, which originate in foci in the body, and (2) exogenous, which gain entrance to the urinary tract by direct implantation. Post-mortem examinations revealed both free stones and incrustations. Their conclusions (6, p. 269) in part are that—

*Proteus ammoniae*, being able to convert urea rapidly into ammonia, precipitated the alkaline earthy salts in the urine, forming chiefly the carbonates and phosphates of calcium and magnesium; these precipitates became attached to the abraded mucous surface. It was noted at the time of the experiments that there was a tendency for these precipitates to break off and form free calculi in the bladder. \* \* \*

Several different investigators have obtained results which indicate that certain dietary deficiencies may be a predisposing cause of calculi. Osborne and Mendel (11) found calcium phosphate calculi prevalent in rats which had been kept on a diet deficient in vitamin A. A study of the diet of these rats showed that "in every instance where calculi developed, the animals were without an adequate source of the fat-soluble vitamin for some time." Fujimaki (3) found that rats fed a diet deficient in vitamin A developed bladder calculi, but when vitamin A was again included in the diet the bladder stones disappeared. Animals fed on a diet deficient in vitamin A, inorganic phosphorus, and calcium formed calculi in a very short time. Bile duct stones took longest to form, and the urinary calculi formed were carbonates. Calculi in animals fed diets deficient in vitamin A or vitamins A and C were phosphatic. McCarrison (8) obtained vesical calculi in rats fed diets characterized by the absence of protein of animal origin, a deficiency of vitamin A, and an excess of earthy phosphates. Stones were found in 29 per cent of the 72 animals used in the experiment, but the stones did not appear until the unbalanced diet had been fed for 56 days. Keyser (7) found that feeding oxamide produced artificial concretions consistently. Oxamide is a foreign crystalloid in the urinary tract. It is precipitated with colloidal material of urine in such a way that fusion of crystals occurs. Concretions are apparently of endogenous origin and are due to defects in metabolism which cause changes in the hydrogen-ion concentration or colloidal content of the urine.

The occurrence of xanthine calculi in sheep has been reported by Easterfield, Rigg, Askew, and Bruce (1). This type is widespread among sheep on poor soils in New Zealand, especially on soils that are deficient in limestone and phosphoric compounds. The greatest incidence of calculus occurred where great deterioration in pasture

had taken place. The pasture grass is very high in nitrogen compounds, the nitrogen ranging from 3.43 to 5.23 per cent. Sheep grown on this pasture are badly stunted, and some of the excess nitrogen in the bladder is deposited in the form of xanthine ( $C_5H_4N_4O_2$ ). Where the poor soils were limed and treated with a phosphate fertilizer and good pastures established, no calculi in the kidneys of sheep were recorded.

Another cause of calculi formation was reported by Tennant (13). He found instances in which the sulphur contained in food was not oxidized and eliminated as sulphates but as cystine. This amino acid, being very insoluble in urine, formed human kidney stone concretions.

The results of Evvard, Culbertson, and Wallace (2, p. 585) indicate that molasses in the diet has some effect on the formation of urinary calculi in feeding lambs. Lots fed beet molasses showed a larger percentage of cases than lots fed cane molasses. Thirty per cent and 48 per cent, respectively, of the lambs in lots fed daily 0.5 pound and 0.7 pound of molasses per lamb had calculi. Evvard et al. believe that "these calculi findings are of practical significance inasmuch as they show quite unmistakably that the feed is a factor in their formation."

#### FREQUENCY OF OCCURRENCE OF CALCULI IN SHEEP

An attempt was made to obtain data on the prevalence of urinary calculi in sheep. For this purpose a questionnaire was sent to 53 prominent sheepmen in different parts of the United States and southeastern Canada. Forty-five replied. Of these only eight had had no calculi trouble in their flocks and did not know of its occurrence. The eight replies came from New Hampshire, New York, Vermont, Massachusetts, Ohio, Virginia, West Virginia, and Texas. However, other replies from four of these States reported cases of calculi. In all, 230 cases, covering a period of from 2 to 15 years, were reported by 40 different men. Twice as many cases of calculi were reported where roots were not fed as where they were. Several instances were mentioned in which rams were fed a ration composed chiefly of roots for several months without any trouble from calculi. Apparently, drinking soft water does not always prevent the formation of calculi, as several cases were reported in sheep that had had only soft water to drink. The occurrence of the trouble was said to be most frequent during the winter months when the ration contained a considerable amount of grain, or when the sheep were being fed heavily on grain for exhibition purposes. However, cases were reported which occurred during the summer when the sheep were on pasture and were being fed grain, and in a few instances, when they were on pasture and received no grain. The rations fed to the sheep affected were composed, in most cases, of the following feeds: Corn, barley, oats, bran, linseed meal, legume hay, and corn silage.

Some sheepmen believe that this trouble is restricted largely to limestone sections. The present writers are convinced that this trouble is not restricted to such regions for they have records of cases which have occurred in regions in which the soil is quite acid. As far as one may judge from these reports it appears that the occurrence of calculi is rare in the New England States, New York, Maryland,

Pennsylvania, Virginia and West Virginia, and that it is relatively common in Indiana, Wisconsin, Iowa, Kansas, Missouri, Oregon, Utah, Colorado, and California.

### OBSERVED CASES OF CALCULI

During the past five years, 12 well-defined cases of calculi have been observed in the Purdue purebred flock, and detailed records of these have been kept. Fifteen western feeding lambs which were brought to the veterinary clinic of the station for post-mortem diagnosis had calculi. They came from four different farms. During the winter of 1929-30, 15 cases were observed among the 224 western feeding lambs being fed experimentally at the Purdue experiment station. Five lambs died from calculi during the feeding period and the remaining 219 were posted when slaughtered. Ten had calculi and in 67 others evidence was found of irritation in the urinary tract which strongly

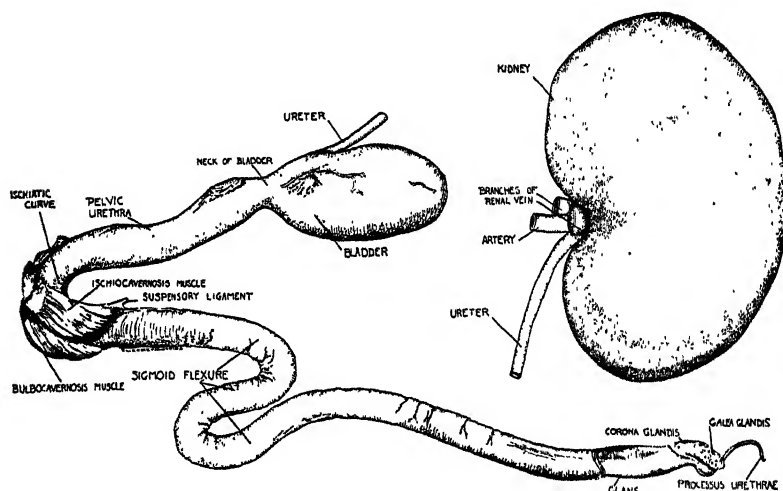


FIGURE 1.—Urinary tract of the male sheep. (After Sisson) (1:2)

suggested either that calculi had been present and had passed out with the urine or that the conditions which prevailed in the urinary tract were conducive to the formation of calculi. However, this was the first time that any appreciable amount of this trouble had been observed in feeding lambs at this station during 17 years of sheep-feeding trials.

The anatomical features of the urinary tract in the male sheep favor the lodgment of calculi or other obstructive bodies in the urethral portion of the tract. There are three places in the penial urethra at which calculi are particularly likely to lodge, namely, the ischial curve, the sigmoid curvature, and the processus urethrae or "worm." The changes in direction and the accompanying narrowing of the lumen of the urethra at the curvatures make it difficult for calculi to pass these points. The small lumen of the processus urethrae is likely to cause the retention of calculi of any appreciable size. (Fig. 1.)

Only a few typical cases observed by the writers will be described. The details vary considerably in individual cases but the major fea-

tures are common to all. The writers have found calculi in every part of the urinary tract, but in only one case were calculi found in every part of the urinary tract in the same sheep.

CASE No. 1 (Purdue No. 1664).—A Rambouillet yearling ram had an attack of calculi in May, 1928, which was a "dry" case. He was operated on but without success and was immediately killed and posted. Calculi were found in both kidneys as well as in the bladder, and the ureters and urethra were nearly filled with small concretions throughout their entire length. Stoppage was complete in both the ischiatic and sigmoid curvatures. The bladder and kidneys were greatly distended with urine, the kidneys being three times their normal size. There was necrosis in the bladder wall extending over an area about 4 cm. in diameter. Rupture of the bladder at this point would evidently have occurred within a few hours. The ram had been fed a mixture of corn and oats, alfalfa hay, and silage during the winter, but had been on pasture for 10 days previous to the attack. He was a rugged, healthy sheep and was not in high condition when stricken.

CASE No. 2 (Purdue 1498).—A 3-year-old Rambouillet ram had an attack on February 24, 1928. When he was first observed, stoppage was only partial, but two days later became complete. An examination of his "worm" was made, and a calculus was found in it. (Fig. 3.) The worm was severed and voiding of urine began immediately. In a few days, recovery was complete.

CASE No. 3 (Purdue 1698).—A 2-year-old Shropshire wether had his first observed attack October 17, 1929. It was not severe, and in three days he appeared to have recovered. On October 23, 1929, he had a second attack which was more severe than the first. The voiding of urine was reduced to a slow seepage. His worm was examined and found to be normal. A probe was inserted into the urethra as far as the first part of the S curvature, but without result. It was decided that the obstruction was so located that there was no hope of removing it. Consequently, the animal was killed and posted. Only one calculus was found in the entire urinary tract. It had lodged in the proximal portion of the sigmoid curvature. During the summer, and until stricken, the wether had run with the ewe flock on pasture; and for several weeks previous to the attack he had been fed one-half pound daily of corn and oats, equal parts.

CASE No. 4 (U. S. D. A. 59 C).—A 3-year-old Southdown ram had his first attack January 1, 1927. Examination revealed small calculi in the worm, but removal of the worm gave only temporary relief. Further examination showed that calculi had lodged in the urethra below the sigmoid curvature. They were located by forcing the penis out of the sheath and pressing the penis between the thumb and first finger. The calculi were forced out of the penis. Voiding of urine began and the ram improved steadily for three days. On the fourth day, however, he showed marked symptoms of pain and 24 hours later died. A post-mortem examination showed a gangrenous condition of the penis, which was evidently caused by forcing the concretions out of the urethra. No calculi were found in the other parts of the urinary tract.

CASE No. 5 (Purdue 1750).—A Hampshire yearling wether showed the first symptoms of calculi July 5, 1929. A large calculus was found about 1 inch above the proximal end of the worm. The worm was inflamed but had not adhered to the penis. It was severed and the calculus was removed. The wether's condition improved, but three days later a second attack occurred in which urination was inhibited. Several doses of formin tablets were administered and the animal recovered. It is by no means certain, of course, that the formin was responsible for his recovery. He had been fed a grain ration of 3 parts oats, 1 part corn since January 1 and had been on rape and bluegrass pasture for three weeks previous to the attack.

#### AUTOPSY REPORT

When the voiding of urine is largely or wholly inhibited for a period of about 48 hours, the bladder or some other portion of the urinary tract usually ruptures. About 24 hours later death occurs. The conditions found in an autopsy of a ram which had calculi, causing the bladder to rupture, are presented below.

The abdominal cavity contained a large amount of bloody urine. There was also a slight amount of fibrinous peritonitis. The scrotum

contained bloody fluid and some clots of fibrin. The fat in the region of the kidneys and the renal lymph glands was infiltrated with fluid. The gall bladder was distended with bile. No other lesions were found outside the genitourinary organs. The ureters were distended with bloody urine and showed the effects of many hemorrhages, particularly near the kidneys. The kidneys were somewhat enlarged, due mainly to distention of the pelvis and calyces by urine. Large hemorrhages had occurred under the lining of the pelvis; and the kidney cortex and medulla contained many petechiae. There was some gross evidence of parenchymatous degeneration in the kidneys. The capsules of the kidneys stripped easily. No calculi were found in the ureters or kidneys.

The urinary bladder was perforated in the dorsal wall, and showed many subserous and submucous hemorrhages. There were many whitish, rather soft calculi and some blood clots in the bladder. Some of the calculi were 1 cm. in diameter. The penial urethra was packed



FIGURE 2.—Typical attitude of a wether showing pronounced symptoms of calculi. (From Easterfield, Rigg, Askew, and Bruce (1))

with calculi from a point at the beginning of the ischial curvature to a point about an inch beyond the beginning of the S curve. The urethra was free from obstruction throughout the rest of its course, but was hemorrhagic throughout its entire length.

#### SIZE AND COMPOSITION OF CALCULI

The cases studied were of two kinds—those in which one or more large stones were present, and those in which large numbers of small stones were present. The stones varied in size, shape, crystalline structure, hardness, and chemical composition. The large ones had relatively larger deposits of mineral salts and were usually harder than the smaller ones. The small ones looked much like fine grains of sand. They contained relatively more organic matter than the large ones, were porous, and generally soft enough to crumble when pressed between the fingers. The concretions were composed of calcium phosphate or aluminum silicate, largely the former, depending on whether grain or hay was most abundant in the ration.

#### SYMPTOMS OF CALCULI

Partial or complete stoppage of the urinary tract is the most positive evidence of calculi. Affected sheep refuse their feed, or eat very little of it, the head is often carried low, the back becomes arched, especially when the animal strains in trying to urinate, and there is a tendency not to exercise. (Fig. 2.)

The animals become dull and listless and, in advanced stages, are very sick. They are inclined to lie down most of the time, usually on their bellies. When they get up, they frequently stamp their hind feet. The wool around the end of the sheath is almost invariably wet. If there is any suspicion that a sheep may have stones, the wool immediately around the end of the sheath should be examined. This symptom is frequently the only reliable one in the early stages.

In Figure 3 is shown a worm with a large calculus in it. This specimen was obtained from a Rambouillet ram, Purdue 1498. Stoppage was complete. The stones in the worm can be felt with the fingers and may be removed in some cases by pressure, but it is generally better to sever the worm near its proximal end. In some cases, the worm becomes attached to the penis. This condition was found in the majority of the cases observed, and evidently favored the lodgment of calculi in the "worm."

#### RECURRENCE OF ATTACKS

The first attack may or may not prove fatal. If the symptoms are recognized soon enough, and if the stoppage is in the worm, the calculi can be removed and recovery follows, provided there is no stoppage in other parts of the urinary tract. In the cases studied by the writers, those that survived the first attack—and a large percentage did—had a second attack, which was always more severe than the first. The number of recoveries was small, partly for the reason that in the Purdue flock a second attack generally meant that the calculi had lodged above the curvature, since the worm was usually removed at the time of the first attack. A few sheep have survived the second attack, but a third attack has proved fatal in every case. One attack is generally followed by a second attack, and in a few cases by a third. Wether lambs are not kept after they have recovered from the second attack.



FIGURE 3.—Calculus lodged in the lumen of the processus urethrae (worm) of a Rambouillet ram

#### PRELIMINARY OBSERVATIONS AND EXPERIMENTS

A survey of the literature showed that published data on the composition and hydrogen-ion concentration of sheep urine are very meager. It was evident, therefore, that it would be necessary to obtain samples of urine from a number of different sheep kept on known rations in order to determine what should constitute normal urine from animals on these rations. This was done, and it was found that the pH values of urine from different individuals ranged from 6.8 to 9.0, depending on the kind of ration fed, and possibly to some extent on



the individuality of the sheep. This finding suggested that a study of the urine might reveal to what extent the ration is utilized in metabolism.

### FEEDING EXPERIMENTS

Feeding experiments designed to show how a certain feed affects the reaction or composition of the urine were formulated. For these tests sheep 1 year old and older were selected from the Purdue flocks. Ewes, wethers, and a few rams were used. A crate 4 by 6 feet having a  $\frac{1}{2}$ -inch mesh-wire bottom and a drain pan was constructed. The mesh-wire bottom was covered with burlap which permitted the collection of both feces and urine. The sheep were fed in dry lots and were prepared for the experiment by being fed the ration to be tested for two weeks prior to the beginning of the experiment.

#### FIRST SERIES

In the first series of experiments three different rations were selected for comparison. Three sheep were placed in each of three different lots. Lot 1 was fed alfalfa hay only; lot 2, corn silage and grain, two parts oats and one part corn; lot 3, mangels and corn stover. The rations were fed for six weeks, and composite samples of urine and feces were taken during this interval. Two sheep were placed in the crate for each run, and the runs were for 24-hour periods. The average daily feed and water consumption per sheep, the amount of urine voided, its pH concentration, its percentage of total solids and ash, and the amount of 0.20 N hydrochloric acid required to neutralize 100 c. c. of urine ash, are given in Table 1.

TABLE 1.—*Influence of the first three rations tested upon the quantity and composition of the urine voided; average per sheep per day*

Lot No.	Daily feed	Average daily water consumption	Average urine voided daily	pH of urine	Total solids of urine	Ash in urine	Quantity 0.20 N HCl required to neutralize 100 c. c. urine ash
		<i>Pounds</i>	<i>C. c.</i>		<i>Per cent</i>	<i>Per cent</i>	<i>C. c.</i>
1	2.87 pounds alfalfa hay	2.79	312	9.0	8.5	5.5	90.6
2	2.6 pounds grain; 1.75 pounds corn silage	1.44	207	7.16	6.7	1.75	.2
3	11 pounds mangels; 0.6 pound corn stover	0	2,310	8.62	1.63	.91	33.2

The data in Table 1 show significant differences in the quantity of urine from the three lots. The large amount voided by the sheep in lot 3 is of particular interest. Twenty-three hundred and ten cubic centimeters per sheep during 24 hours is a very large output. It should be noted that the sheep in this lot drank no water. The differences in the pH values, the percentage of ash, and the amount of 0.20 N HCl required to neutralize the water-soluble bases in the ash, are of especial interest in connection with the problem under consideration.

The urine from the sheep in lot 1 was very alkaline when voided, having a pH value of 9.0 to 9.2. It contained 8.5 per cent of total solids, 5.5 per cent of ash, and required 90.6 c. c. of 0.20 N HCl to neutralize the water-soluble bases in the ash of 100 c. c. of urine. These data indicate that an abnormally large quantity of nutrient

material was being eliminated by way of the kidneys, and the large ash content might suggest a tendency to form calculi, but it was mostly water-soluble and there was no evidence of the formation of any gravel by combination with the potassium or other elements present.

The sheep in lot 2 produced what appeared to be more normal urine, having a pH value of 6.9 to 7.3 with a volume of 225 c. c. per sheep in 24 hours. The total solids averaged 6.7 per cent and the ash 1.75 per cent. That the ash material contained only traces of soluble bases is shown by the fact that only 0.2 c. c. of 0.20 N HCl was required to neutralize 100 c. c. of urine ash. This would indicate that the soluble bases in the feed were neutralized by acid radicals—phosphates, silicates, and sulphates—contained or produced by the feed.

The fresh urine from lot 3 had a pH range of 8.4 to 8.8. It contained only 1.63 per cent of total solids and 0.91 per cent ash. The relatively large amount of 0.20 N HCl required to neutralize the alkalinity of the ash of this very dilute urine appears to the writers to be very significant. It was due largely to the presence of compounds of potassium in relatively large amounts. Since this element does not form many compounds which are insoluble in water, it is not probable that the cause of calculi formation is the presence of beets in the ration, but rather that the beets, so far as they are concerned, may serve to prevent the formation of calculi through the production of water-soluble in place of water-insoluble compounds.

Many cases of calculi have been reported in sheep which were being fed roots, particularly mangels, but the rations fed in these cases almost invariably contained, in addition to the roots, legume hay and grain. Roots may be a contributing factor when fed along with hay and grain, for the calcium in the roots may combine with the phosphorus in the grains to form calcium phosphate which is the chief component of most of the calculi analyzed by the writers. This is an admission that roots may be a contributing factor under certain conditions, but not that roots are a primary cause of calculi formation. The fact that calculi are formed when the ration does not contain roots and when sheep have never been fed roots, leads the writers to believe that their occurrence is not wholly dependent, if at all, on the presence of roots in the ration.

The replies to our questionnaire mentioned showed that its occurrence was more frequent when the ration did not contain roots. The present studies lead us to believe that calculi formation is impossible when roots only are fed, and that they are not likely to be formed when the ration is composed of roots and legume hay, or roots and grain.

The three lots of feeds used in these experiments represented widely different mineral balances. The potassium compounds predominate in the basic material in beets, making the urine ash alkaline, as there are no acid-producing compounds present in quantity with which to combine. The alfalfa hay ration is outstanding in its calcium content but low in phosphorus and sulphur. Hence, the urine ash is very alkaline. The grain and silage ration is low in basic materials and high in silicates and phosphates. Hence, the urine ash is usually neutral or even slightly acid.

## SECOND SERIES

In a second series of experiments the study was extended to include a number of different combinations of feeds.

These are shown in Table 2.

TABLE 2.—*Influence of various rations fed upon the quantity and composition of urine voided by sheep*

Ration	Average amount of urine voided	Total solids in urine	Quantity of 0.20 N HCl required to neutralize 100 c. c. urine ash	Ash in urine	pH of urine	Crystals formed in standing urine
	C. c.	Per cent	C. c.	Per cent		
Rape pasture.....	a 529	5.44	16.5	3.71	7.5-8.5	None.
Bluegrass pasture.....	a 520	3.3	10.0	2.90	7.6	Do.
Alfalfa hay, 2 pounds; oats, 1.5 pounds.....	a 483	3.91	22.3	1.10	7.8-8.6	Few.
Alfalfa hay, 2 pounds, corn, 1 pound.....	b 275	4.38	17.8	1.48	7.8-8.2	Do.
Bluegrass pasture, mixture of 3 parts oats and 1 part corn, 1.5 pounds.....	a 330	3.91	11.0	1.52	7.5-7.7	None.
Bluegrass, mixture of 2 parts corn, 2 parts oats, 1 part bran, 1.5 pounds.....	a 262	4.5	7.4	2.1	7.6-8.0	Few.
Alfalfa hay, 2 pounds, mixture of 2 parts corn, 2 parts oats, and 1 part bran, 1.5 pounds.....	a 290	4.61	29.0	1.45	8.3	Many.
Alfalfa hay, 2 pounds; bluegrass; mixture of 3 parts oats and 1 part corn, 1.5 pounds	a 350	4.51	29.3	1.69	8.1-8.8	Few.
Corn silage, 2.5 pounds; mixture of 1 part corn and 3 parts oats; 1 pound.....	b 118	4.30	0.10	1.69	7.3	None
Corn silage, 2.5 pounds; mixture of 3 parts oats and 1 part bran, 1.75 pounds.....	b 178	6.96	0.9	1.01	6.8-8.0	Many.
Corn silage, 1.2 pounds; clover hay, 1.6 pounds.....	b 320	4.1	27.4	1.90	8.5	Solids, not crystals.
Rape pasture; mixture of 3 parts oats and 1 part corn, 2 pounds.....	a 272	5.590	2.72	2.39	8.3	Many.
Rape and bluegrass pasture; mixture of 3 parts corn and 1 part oats, 0.5 pound.....	b 410	5.41	2.72	2.54	7.8-8.6	Few.

a In 12 hours.

b In 24 hours.

## RAPE V. BLUEGRASS PASTURE

Since rape pasture is often provided for sheep, this feed was used for study in contrast with bluegrass pasture, which is usually rated well whenever it can be obtained. It was found that the sheep fed rape produced somewhat more basic urine than those fed bluegrass. Urine from the former contained 5.44 per cent total solids, and 16.5 c. c. of 0.20 N HCl were required to neutralize the ash; whereas the urine from sheep fed bluegrass contained 3.3 per cent total solids, and 10.0 c. c. of HCl were required to neutralize the ash.

This difference might not seem great enough to be significant, but it was noticed that when 2 pounds of grain were fed the rape urine ash was neutralized and the urine deposited crystals upon standing for several days. This indicates that the rape contained more calcium, magnesium, phosphate, and silicate compounds than the bluegrass. The latter gave no crystal deposits when the urine stood in a flask for a month, and the addition of grain to the ration did not yield a neutral urine ash. On the contrary 11.0 c. c. of 0.20 N HCl per 100 c. c. of urine were required to neutralize it. This alkalinity was due largely to the production of potassium carbonate in the formation of the ash material.

## ALFALFA—OAT RATION

When grain was fed with alfalfa hay in the proportion of 2 pounds of hay to 1.5 pounds of oats, 183 c. c. of urine per sheep were produced in 12 hours. This urine had an average pH value of 8.2, contained 3.91 per cent of solids and 1.10 per cent of ash. In this case the calcium, potassium, and other bases of the alfalfa were more nearly neutralized by the phosphoric acid from the oats, and only 22.3 c. c. of 0.20 N HCl were required to neutralize the ash from a 100 c. c. sample. Two other sheep on the same ration produced 310 c. c. of urine each, which had the same pH value and contained 3.7 per cent of solids and 1.26 per cent of ash. The urine ash was less basic than before, and only 16.4 c. c. of 0.20 N HCl were required for the ash from a 100 c. c. sample.

The low percentage of solids (3.7 per cent) in the urine would indicate an efficient utilization of the food nutrients; besides, the alkalinity was not high enough to be irritating.

## OATS, CORN, AND BLUEGRASS PASTURE RATION

When bluegrass pasture was substituted for the alfalfa hay and 1.5 pounds of grain (consisting of 3 parts oats and 1 part corn) were fed, the large quantity of potassium ( $K_2O$ ), common to most pastures, averaged nearly half of the total ash content, yet gave the urine ash a less basic reaction than alfalfa and oats. The urine output was 330 c. c. per sheep in 24 hours, contained 3.91 per cent total solids, 1.52 per cent ash, and 11.0 c. c. of 0.20 N HCl were required to neutralize the ash from 100 c. c. of urine.



FIGURE 4.—Crystals obtained from sheep urine that had stood for 24 hours

Since the ash was quite water soluble, it was hardly to be expected that calculi would be formed on a grain and bluegrass pasture ration. Reports from experienced sheepmen indicate that calculi rarely, if ever, form when bluegrass pasture constitutes an appreciable part of the ration.

## ALFALFA, GRAIN, AND BRAN RATION

When wheat bran is introduced into a ration it carries with it a large mineral content, especially phosphorus compounds. These readily form water-insoluble combinations with calcium, magnesium, and aluminum, and gravel is deposited in the kidney and bladder, thereby often causing serious trouble to male sheep. The gravel tends to combine to form a conglomerate either in the kidneys, the ureters, the bladder, or the urethra, which makes it impossible for the urine to be voided. When bran is fed with alfalfa hay, deposits of calcium phosphate, ammonium oxalate, and aluminum phosphate,

etc., form in the urine a few hours after it has been passed. A photograph of crystals obtained from urine which had stood for 24 hours is shown in Figure 4.

These crystals often have a definite structure and are quite easily distinguished from the organic solids always present in urine. The crystals are heavier and readily settle to the bottom of the flask, whereas the organic *débris* stays in suspension longer, thus making it possible to obtain an estimate of the amount of each. When sheep are on pasture there is no evidence of such solids in the urine, but upon the addition of grain, especially wheat bran, even to a pasture ration, there will be a tendency for gravel to form when the urine stands a day or more. This was noticed especially in the case of sheep on rape pasture. As long as the sheep were kept on rape no crystals appeared in the urine, but when corn and oats were given in addition, gravel formation was noticed in all samples. About half of the deposit was mineral material, chiefly calcium phosphate and aluminum silicate. This study of the relation between the urinary ash of sheep and different feeds is being continued for the purpose of finding a ration that will be most effective in preventing calculi formation.

#### SILAGE AND GRAIN RATION

When corn silage was fed with a grain mixture of corn and oats, the urine ash was nearly neutral. When alfalfa or clover hay was fed with silage and grain, the urine ash was more alkaline than when silage and grain were fed. When the ration consisted of mangels or alfalfa hay (Table 1) the urine ash was strongly alkaline. Mangels have an effect on the reaction of the urine ash just the opposite to that of silage.

The reaction of the urine is further affected by the rate at which urease enzymes convert urea to ammonium salts. Williard<sup>4</sup> has shown that the rate of crystal growth and the size of the crystals formed are affected by the rate of the urease reaction. When urea was used for this purpose instead of ammonium hydroxide, the precipitation was granular. The authors believe that their results warrant the hypothesis that the retention of urine in the bladder is conducive to the formation and growth of crystals. When the ration fed is composed of feeds which produce insoluble mineral compounds and is lacking in succulence, and when the atmospheric temperature is low, the urine becomes very concentrated and is likely to be retained longer in the bladder. This gives urease sufficient time to form ammonium salts which in turn increases the alkalinity of the urine to a point where certain insoluble compounds are precipitated. The crystals may become large enough to close the urinary tract.

#### SUMMARY

The replies received from a questionnaire sent to sheepmen indicate that the occurrence of calculi in sheep is fairly general in the United States, but is more frequent in some sections than in others.

Twelve positive cases of calculi were observed and studied by the writers, and 25 autopsies were performed. Post-mortem data on 219 lambs were obtained. Ten of these lambs had calculi.

Calculi were found in the kidneys, the ureters, the bladder, and the urethra, but in only a few cases were they found in all of these parts in the same sheep.

<sup>4</sup>WILLARD, H. H. Information to the authors.

The calculi obtained and analyzed were composed largely of calcium phosphate and organic matter. They varied widely in size and in degree of hardness.

Calculi were found more often in the processus urethrae (worm) than in any other part of the urinary tract. The S-curvature of the urethra in male sheep offers a mechanical difficulty which frequently prevents the passage of calculi beyond this point. This is the place where large calculi are most likely to lodge and cause complete stoppage.

Partial or complete inability to urinate is the most positive symptom of calculi formation. When calculi are present the wool around the end of the sheath is invariably wet. In severe cases, the back becomes arched because of the great effort required to urinate.

If the calculus is in the worm, and the worm is removed, recovery is rapid. The writers do not know of a successful method of dislodging large calculi from the S-curvature of the urethra.

It was found that one attack was likely to be followed by a second or a third attack. In no instance did the animal survive the third attack.

Experiments were made to determine how different rations would affect the reaction or composition of urine. These experiments were conducted largely with wethers and ewes 1 year old and older, and covered a period of over two years. Forty-two different sheep were used and 104 samples of urine were collected.

When roots were fed, 2,200 to 2,500 c. c. of urine per sheep were produced in 24 hours, whereas from sheep on dry feed the average production was only 200 to 250 c. c., but pasture increased this amount to about 600 c. c. per half day.

The reaction of the fresh urine varied from pH 6.8 to 9.0, depending on the feed of the animal. Sheep on alfalfa or beet rations produced a very basic urine ash, but when a cereal grain or bran was added to the ration the reaction of the urine was nearly neutral. The presence of the enzyme urease in urine also is responsible for it becoming basic rapidly upon standing due to the formation of ammonium carbonate from urea. This accounts for part of the large deposits of organic residues formed as the enzyme action continues making the urine much more basic.

When the ration was utilized to best advantage by the sheep, about 3 per cent of solids were found in the urine. On the most undesirable rations the sheep produced a urine containing 6 to 8 per cent of solids, which indicates a large waste of nutrient material. Moreover, the urine ash was highly basic. It seems probable that such urine would irritate the urinary tract and thus account for the observed cases of marked inflammation of the urethra in the absence of calculi. When sheep were fed alfalfa or clover hay or roots alone a highly basic urine was produced.

Certain mineral compounds are sometimes precipitated from the urine and the crystals cluster around an organic nucleus, which results in the formation of calculi.

The nature of the urine affords an important guide to the utilization of a feed and has a notable effect on the production of calculi.

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## EARLY DEFLOURATION AS A METHOD OF INCREASING COTTON YIELDS, AND THE RELATION OF FRUITFULNESS TO FIBER AND BOLL CHARACTERS<sup>1</sup>

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### INTRODUCTION

A study of the growth reactions of the cotton plant in Arizona during the seasons of 1926 and 1927 indicated that it might be possible, at least under some conditions, materially to increase cotton yields by artificially delaying for several weeks the normal initiation of boll setting. The premises for this view were briefly as follows: Experiments had shown that an increased vegetative growth, an increased rate of floral-bud formation, and a decreased shedding rate followed when all previously set bolls were removed from cotton plants. These results,<sup>3</sup> which were generally confirmed by Murneek's results with the tomato,<sup>4</sup> indicated in addition that plants so treated carried a heavier boll load, in proportion to their size, soon after defruiting than did untreated control plants. This latter observation, which can not be fully explained, is considered as having had an important bearing on the outcome of the experiments herein reported. The effect of defruiting on the rate at which new bolls are set is shown graphically in Figure 1. During the 36-day interval between the first and second defruiting the 25 control plants increased the number of bolls carried at an average rate of 3.5 bolls per day; whereas the defruited plants increased the number of bolls carried at the rate of 10.7 bolls per day. Other experiments showed, also, that a more extensive root system was developed by plants grown without bolls, or with a limited number of bolls during the early part of the season, than by normally fruited plants. The nutritional dominance of bolls over the vegetative growth of the cotton plant had frequently been observed to inhibit boll setting and terminal bud and branch development. A reduction in growth rates following the initiation of flowering and boll setting was a common observation. On the basis of general physiological grounds it was recognized that the number of bolls that can be developed by a cotton plant is not only relative to the favorableness of environmental conditions, among other factors, but is also directly related to the leaf area of the plant. In view of these facts and indicated relationships, it seemed logical to assume that an increased yield would result if the first bolls set by cotton plants were sacrificed in favor of a larger plant with a greater initial photosynthetic capacity.

The extent to which cotton yields could be increased by this method were considered as being dependent upon two primary but

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undetermined factors: (1) The length of time that the initiation of boll setting should be delayed in order to gain the most advantageous effect, and (2) the extent to which boll development is dominant over vegetative growth in different varieties of cotton. The conditions that determine such dominance are imperfectly understood, but it is known that different varieties may behave quite differently

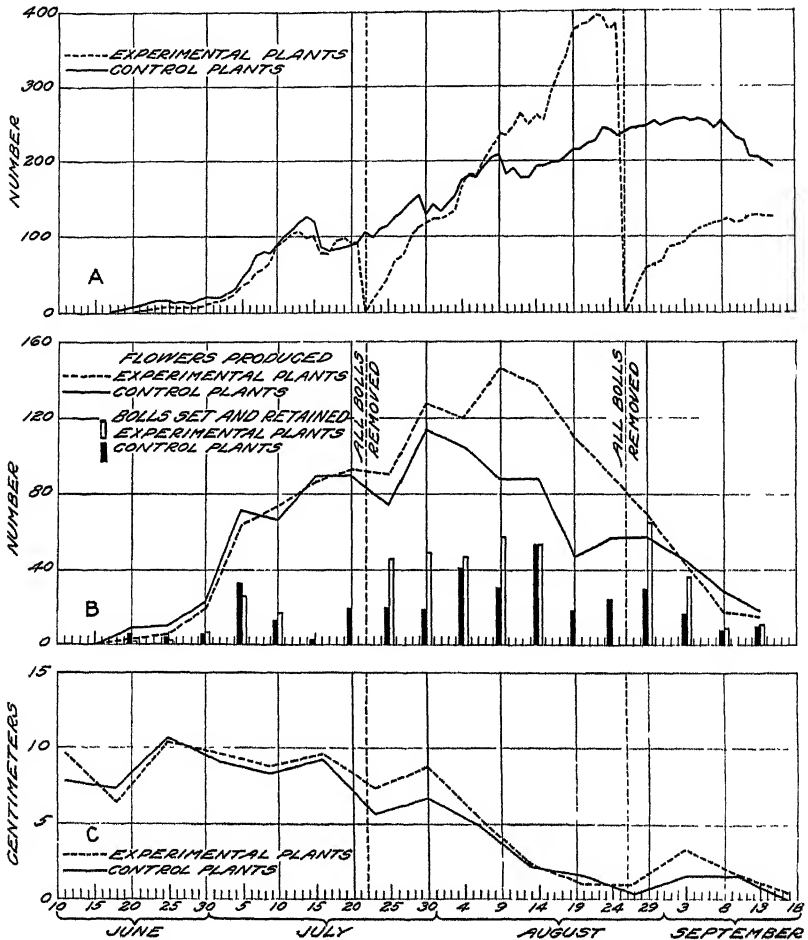


FIGURE 1—Effect of defruiting, i. e., removing all bolls, on (A) the daily number of developing bolls carried by 25 Acala cotton plants, (B) the number of flowers produced and the number of bolls set and retained from these flowers on 25 plants, and (C) the weekly elongation rates. In each graph the defruited (experimental) plants are compared with untreated (control) plants

in different localities and to a lesser extent in different seasons. Presumably, a determinate cotton variety, i. e., one that tends to set a heavy crop of early bolls, should give the most outstanding response to this treatment; whereas a variety inclined to make a rank growth early in the season might benefit little or not at all. The Acala strain used in experiments herein reported has proved to be less determinate in its growth and better suited to Arizona conditions than other strains with which it has been compared.

The experimental plans for testing the possibility of increasing yields by delayed boll setting were so arranged that data would be supplied on the effects of the treatments on boll characteristics and on the quality of fiber produced. The delayed initiation of boll setting was accomplished by removing all flowers from experimental plants during the early part of the flowering period. To augment the data on the effect of plant load on boll and fiber characters, a second treatment was included to make possible observations on the behavior of sparsely fruited plants. This latter purpose was accomplished, with other plants, by limiting boll setting to the first node of any fruiting branch.

The present paper, therefore, is a report of an investigation on the effect upon yields of delayed boll setting and on the effect upon the bolls and fiber of treatments resulting in plants that were either more heavily or more lightly fruited than untreated control plants.<sup>5</sup>

Florists and horticulturists have recognized for many centuries that when only a few of the flowers or fruits on a plant are left to develop, a superior product may be expected. The characters of the bolls and fiber of a particular variety of cotton are relatively stable, and the effect of the difference in the number of bolls borne by otherwise similar cotton plants upon boll size and the character of the ginned cotton has not been previously determined. It was desirable that this important relation should be worked out; and in connection with the proposed method for increasing yields it seemed especially desirable, if not necessary, that the effect of plant load on fiber character should be determined.

The difference in nutritional relationship between bolls on sparsely and on heavily fruited plants must, in a large measure, be comparable to the differences which are brought about by changes from favorable to unfavorable environmental conditions, since in either case the competition among the growing bolls for available nutrients is of primary importance. Therefore, to the problem of the effect of adverse climatic and soil conditions upon boll and fiber characters a partial solution is suggested by the data herein presented.

The greater number of the observations preceding the present experimental investigation had been made on the Acala upland variety. The Pima Egyptian cotton is very different from upland cotton, not only in its morphology but also in many of its physiological characteristics and growth reactions. For these reasons uncertainty was felt as to what effect early defloration would have on Pima yields. The same treatment was applied to each variety, and, as will be noted later, the early-deflorated Pima plants did not set a heavier crop until late in the season. The Pima plant produces a smaller boll than the Acala and is much less determinate in its growth. Under field conditions an early setting of bolls on Pima plants does not retard growth and cause flowers to shed to the extent that it does in Acala.

#### EXPERIMENTAL METHODS

The experimental plants, both Acala upland and Pima Egyptian, were grown in plot D-1-17, United States Field Station, Sacaton, Ariz., during the season of 1928. The plot was 200 feet long and was

<sup>5</sup> Special acknowledgment is due Carl A. Moosberg for the immediate care of the experiments, for the greater portion of the measurements, and for aid in the tabulation of the results.

planted with four rows of Acala and three rows of Pima. The rows were 42 inches apart, and the plants were thinned to 15 inches apart in each row.

From each branch of 75 plants of each variety all flowers or floral buds of large size appearing at fruiting-branch nodes beyond the first were systematically removed throughout the flowering season. In tables and discussions these plants will be referred to as the 1-boll-per-branch plants. A number of the first nodes of the fruiting branches, particularly in the Acala variety, failed to develop a boll; as no other flower had been left to develop, such branches remained barren.

In an adjacent row, in both the Acala and Pima sections, all flowers or floral buds of large size were systematically removed from 25 plants until July 25, after which date the flowering and boll setting of the plants were not interfered with. These plants, each third plant of the row, will be referred to as the early-deflorated plants. The 50 plants intervening in pairs between the consecutive early-deflorated plants were reserved as control plants.

In this experiment the flowering season was divided into three periods: (1) July 1 to August 4, (2) August 5 to August 22, and (3) August 23 to September 15. On August 4 all flowers and previously set bolls on all the plants were tagged with blue tags. From August 5 to August 22, inclusive, an 18-day period, all flowers were marked with dated white tags as they appeared, and flowers that appeared subsequently remained untagged. If bolls from the middle or white-tag period developed, the date of opening was also recorded on the tag. As soon as any boll of this experiment opened it was inclosed in a paper bag to protect the seed cotton and prevent its loss. All vegetative branches were removed from the plants as they appeared, thus leaving only the main axes and fruiting branches.

A considerable portion of the information reported herein was derived from measurements made on 100-boll samples. These samples were selected at random after each crop was gathered but before the bolls were removed from their individual paper bags.

Very few flowers appeared after the middle of September on any of the experimental plants. Flowers set at a later date do not contribute materially to the yield of cotton in Arizona. In this experiment the last cotton was picked on November 13, following frosts which occurred during the second week in November. In a supplementary experiment, which will be described later in this paper, the last picking was made on January 3; but the bulk of the cotton from that experiment was picked on October 10. The vegetative branches were not removed from the plants in the supplementary experiment.

## EXPERIMENTAL RESULTS

### NUMBER OF MATURE BOLLS AND WEIGHT OF FIBER AND SEED

In Table 1 the effects of the various treatments upon the two varieties are given in terms of the number of bolls matured and the quantity of fiber and seed produced per plant from the flowers appearing during the three periods into which the experiment was divided. The ratios of weight of fiber to weight of seed are also given.

By the end of the first period the Acala 1-boll-per-branch plants had set approximately half as many bolls as the control plants,

whereas the early-deflorated plants had set approximately 10 per cent more bolls than the control plants.

TABLE 1.—*Mean number of mature bolls and mean weight of fiber and seed per plant in Acala and Pima cotton, from flowers of each period of season (July 1 to September 15, 1928) for each of three treatments*

Period and treatment	Acala				Pima			
	Mean bolls per plant	Mean weight of fiber	Mean weight of seed	Ratio of fiber weight to weight of seed	Mean bolls per plant	Mean weight of fiber	Mean weight of seed	Ratio of fiber weight to weight of seed
First period (from flowers of July 1 to August 4):	<i>Number</i>	<i>Grams</i>	<i>Grams</i>		<i>Number</i>	<i>Grams</i>	<i>Grams</i>	
1 boll per branch.....	7.33	18.5	35.5	0.520	11.45	8.9	26.8	0.332
Control.....	14.14	33.8	62.9	.538	28.02	24.3	58.2	.418
Early deflorated.....	15.68	36.0	66.2	.544	13.96	13.1	32.6	.402
Second period (from flowers of August 5 to 22):								
1 boll per branch.....	2.16	5.3	10.6	.500	3.17	3.2	8.6	.372
Control.....	2.16	4.8	9.5	.505	10.70	8.7	22.0	.395
Early deflorated.....	4.40	9.6	16.8	.571	21.08	19.9	48.7	.409
Third period (from flowers of August 23 to September 15):								
1 boll per branch.....	1.20	2.8	5.7	.492	1.95	3.1	7.5	.414
Control.....	.50	1.0	2.2	.455	3.64	3.9	9.7	.402
Early deflorated.....	0	0	0		14.88	13.2	28.1	.470
Total or average for season:								
1 boll per branch.....	<sup>a</sup> 10.7	26.6	51.8	.514	16.6	15.2	42.9	.354
Control.....	<sup>a</sup> 16.8	39.6	74.6	.531	42.4	36.9	89.9	.410
Early deflorated.....	<sup>a</sup> 20.1	45.6	83.0	.550	49.9	46.2	109.4	.422

<sup>a</sup> All values in this table, except the number of bolls per plant in Acala, were derived from the gross yields of the respective treatments and periods. The number of bolls on each of the Acala plants was counted separately and the probable errors for the season are as follows. One boll per branch,  $10.70 \pm 0.23$  control,  $16.8 \pm 0.36$ ; deflorated to July 26,  $20.1 \pm 0.50$ . The difference between the control and early-deflorated Acala is  $3.3 \pm 0.62$ .

Boll setting during the second period was comparatively light in Acala for each of the three treatments, but the early-deflorated plants set more bolls than either the control plants or the 1-boll-per-branch plants. By the end of this period the early-deflorated plants had set 23 per cent more bolls than the control plants.

Early defloration (Table 1) resulted in a larger crop in both the Acala upland and Pima Egyptian varieties, irrespective of whether the difference is measured in terms of the number of mature bolls or the weight of fiber. The differences in the ratios of fiber to seed indicate that early defloration increased the weight of fiber per plant relatively more than the weight of seed.

#### GROWTH OF PLANTS AND NUMBER OF FIRST-NODE BOLLS RETAINED

Detailed measurements were made on the Acala upland plants at the end of the season, but only the height of the Pima plants was recorded. Table 2 shows that the plants of both varieties attained greater height when boll setting was limited to the first node of each branch. Early defloration stimulated the vegetative growth of both the Acala and the Pima plants during the first part of the flowering period; and the Pima plants were still growing more rapidly than the controls as late as September.

TABLE 2.—Mean height of *Acala* and *Pima* plants at end of season (November 13, 1928), and mean number of fruiting branches and first-node bolls per plant on *Acala*

ACALA			
Treatment	Mean height	Mean first-node bolls on fruiting branches	Mean fruiting branches
	Inches	Number	Number
1 boll per branch.....	47.9	10.7	24.5
Control.....	41.7	5.5	22.6
Early deflorated.....	45.5	* 2.6	24.1

PIMA			
1 boll per branch.....	62.8		
Control.....	50.2		
Early deflorated.....	57.8		

\* Many of the flowers at the first nodes of these plants were removed during the early-defloration period.

The *Acala* 1-boll-per-branch plants produced approximately twice as many bolls on the first nodes of the fruiting branches as did the control plants. The number of bolls on the first nodes of the fruiting branches of the early-deflorated plants was low, since many of the flowers at these positions had been removed during the early-defloration period.

#### MEAN WEIGHT OF BOLLS AND BURS, SEED COTTON, FIBER, AND SEED PER BOLL

In the upland variety (*Acala*) the comparisons of the boll characters from flowers appearing during the second period of the experiment were looked upon as furnishing the best data for the determination of the influence of the number of bolls per plant on boll characters. Bolls from the second-period flowers in the different treatments developed under the greatest extremes with respect to the total load on the plants and therefore with respect to competition between bolls. Similar relations existed between the bolls of the different treatments set during the first period, but the bolls of the early-deflorated plants were all set during the last 10 days of that 35-day period, and a seasonal factor was therefore introduced. In considering the results of the *Acala* upland comparisons it should be borne in mind that the bulk of the crop was from first-period flowers. The *Acala* early-deflorated plants produced no bolls from third-period flowers, whereas the similarly treated *Pima* plants produced almost a third of their crop from these flowers.

The total load on the early deflorated *Pima* plants (Table 1) did not equal the load on the control plants until the last period, and as a result, in terms of plant load, the early-deflorated *Pima* plants occupy a position between the 1-boll-per-branch and the control plants. Early deflorations of the *Pima* plants greatly stimulated vegetative development, and, relative to plant size or leaf area, the boll load on the early-deflorated *Pima* plants was probably always less than that on the control *Pima* plants. The load differences between the control and 1-boll-per-branch plants, however, were quite marked throughout the season.

TABLE 3.—Mean weight of bolls and burs and of seed cotton, fiber, and seeds per boll, in Acala and Pima cotton, from flowers of each period of season (July 1 to September 15, 1928), for each of three treatments

[Data are based on 100-boll samples]

Period and treatment	Acala					Pima				
	Mean weight of open bolls	Mean weight of burs	Mean weight of seed cotton	Mean weight of fiber <sup>a</sup>	Mean weight of seed <sup>a</sup>	Mean weight of open bolls	Mean weight of burs	Mean weight of seed cotton	Mean weight of fiber	Mean weight of seed
First period (from flowers of July 1 to Aug. 4):										
1 boll per branch.....	Gms. 10.79	Gms. 2.82	Gms. 7.97±0.071	Gms. 2.66	Gms. 5.11	Gms. 4.76	Gms. 1.36	Gms. 3.40±0.042	Gms. 0.94	Gms. 2.41
Control.....	9.17	2.12	7.05±.074	2.38	4.49	4.34	1.05	3.29±.038	.89	2.25
Early deflorated.....	8.72	2.13	6.59±.088	2.23	4.30	4.32	1.16	3.16±.013	.83	2.11
Second period (from flowers of Aug. 5 to 22):										
1 boll per branch.....	10.62	3.17	7.45±.092	2.43	4.84	5.26	1.74	3.52±.041	.97	2.55
Control.....	8.87	2.18	6.69±.081	2.21	4.42	3.90	1.02	2.88±.039	.79	2.06
Early deflorated.....	8.65	2.14	6.51±.109	2.25	4.01	4.54	1.19	3.35±.045	.89	2.23
Third period (from flowers of Aug. 23 to Sept. 15):										
1 boll per branch.....	-----	-----	-----	-----	-----	5.64	1.83	3.81±.042	1.05	2.57
Control.....	-----	-----	-----	-----	-----	3.64	.98	2.66±.047	.79	1.84
Early deflorated.....	-----	-----	-----	-----	-----	3.95	.97	2.98±.057	.88	1.90

<sup>a</sup> Owing to losses in ginning, including foreign material and changes in moisture, the combined weights of fiber and seed do not equal seed cotton per boll.

The bolls of the Acala 1-boll-per-branch plants (Table 3) averaged, respectively, 18 and 20 per cent heavier for the first and second periods than did those of the control plants. The difference between the mean weight of individual bolls on the control and on the early-deflorated plants in the two periods was 5 and 2 per cent, respectively, the bolls being slightly smaller on the early-deflorated plants.

The burs were 33 and 45 per cent heavier on the 1-boll-per-branch Acala plants in the two respective periods, but only a small difference is shown between the burs of the control and the early-deflorated plants. (Fig. 2.)

In Acala the weight of the seed cotton per boll for the three treatments also stands in inverse order to the number of bolls per plant, which was greatest on the early-deflorated plants and least on the 1-boll-per-branch plants. The mean seed-cotton weight per boll on the 1-boll-per-branch plants exceeded that of the control plants by 13 per cent in the first period and by 11 per cent in the second. There was less seed cotton per boll on the early-deflorated plants than on the control plants. Early defloration resulted in a marked decrease in weight of seed in bolls from the second period, but the weight of fiber per boll was not decreased. The weight of fiber and seed per boll was distinctly greater for the bolls from the 1-boll-per-branch plants than for the other treatments.

In the Pima variety (Table 3), limiting bolls to the first nodes materially increased the weight of the individual bolls and of the burs, seed cotton, seed, and fiber per boll. The greatest differences shown by this variety were in the bolls from the third-period flowers. The mean weight of seed cotton per boll was 43 per cent greater and the weight of fiber per boll 33 per cent greater on the 1-boll-per-branch plants than on the controls. Of the increases in weight of the various parts of the boll, that of the bur was greatest, being 86 per cent.

The weight of control Pima bolls and of the different boll fractions decreased as the season progressed, whereas those of the 1-boll-per-branch plants increased.

A cessation in growth of the control Pima plants was quite noticeable in late August and September. The early-deflorated Pima plants, however, continued their growth throughout the late summer.

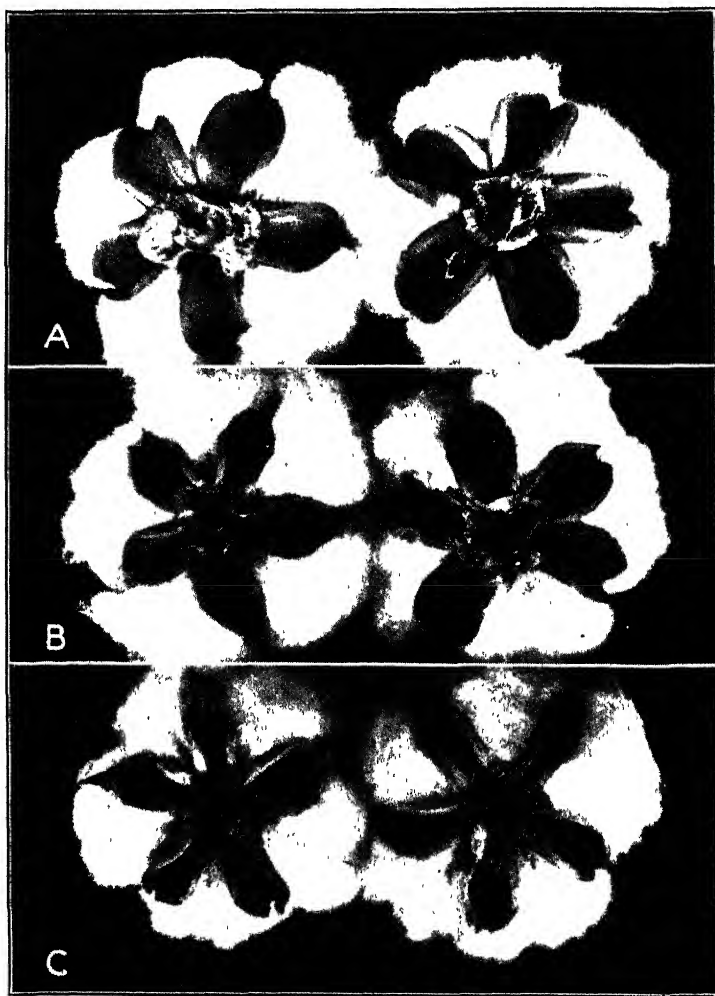


FIGURE 2.—Open Acala bolls from flowers of the second period.  $\times$  about  $\frac{1}{2}$ . A, From the 1-boll-per-branch plants. The enlarged burs are to be noted particularly. B, From the control plants. C, From the early-deflorated plants

Table 3 shows the differences between the weight of bolls and boll components of the control and of the early-deflorated Pima plants as being somewhat uncertain during the first period. During the second and third periods, however, the values for the bolls and boll components of the early-deflorated Pima plants are definitely larger in all measurements, except those for third period bolls, than those of the

control plants. The control Pima plants were considered as being more heavily laden with respect to their size than the early-deflorated plants.

In general, the weight of the burs was influenced to a greater extent by the treatments than the weight of the seed cotton, and the weight of the seed to a greater extent than the weight of the fiber. (See also ratios in Table 1 and seed weights in Table 5.)

#### NUMBER OF LOCKS PER BOLL, SEEDS PER LOCK, AND IMPERFECT SEEDS

If nutritional factors influence the number of locks per boll, and it seems logical that they should, one would expect to find a greater number of locks in bolls on the 1-boll-per-branch plants than in bolls on the control plants, particularly in bolls from the third-period flowers. The number of locks per boll must be determined at a very early stage in the development of the buds. The data (Table 4) do not show significant indications of a nutritional relationship, though many of the differences are in the directions that might be expected.

TABLE 4.—Percentage of 5-lock and 4-lock bolls for Acala and Pima, respectively, and mean number of seeds per lock and of notes and imperfect seeds per boll in Acala and Pima cotton, from flowers of each period of season (July 1 to September 15, 1928), for each of three treatments

[Data are based on 100-boll samples]

Period and treatment	Acala			
	5-lock bolls	Mean seeds per lock	Mean notes per boll	Imperfect seeds per boll
First period (from flowers of July 1 to Aug. 4):	<i>Per cent</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>
1 boll per branch.....	91±1.9	7.27±0.082	11.41±0.316	-----
Control.....	86±2.4	7.36±.090	10.73±.442	-----
Early deflorated.....	87±2.3	7.04±.098	11.40±.355	-----
Second period (from flowers of Aug. 5 to 22):				
1 boll per branch.....	75±2.9	7.24±.105	9.48±.350	1.08±0.147
Control.....	74±3.0	7.63±.085	6.95±.237	1.43±.234
Early deflorated.....	70±3.1	7.07±.114	8.30±.297	3.38±.412
Third period (from flowers of Aug. 23 to Sept. 15):				
1 boll per branch.....	<sup>a</sup> 50±3.6	7.44	9.42	.77
Control.....	<sup>b</sup> 48±6.7	7.52	6.2	1.80
Early deflorated.....				

Period and treatment	Pima			
	4-lock bolls	Mean seeds per lock	Mean notes per boll	Imperfect seeds per boll
First period (from flowers of July 1 to Aug. 4):	<i>Per cent</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>
1 boll per branch.....	10±2.0	6.19±0.069	3.90±0.136	-----
Control.....	16±5.5	6.12±.071	4.20±.145	-----
Early deflorated.....	6±1.6	5.84±.084	4.50±.147	-----
Second period (from flowers of Aug. 5 to 22):				
1 boll per branch.....	5±1.5	6.18±.077	3.26±.175	0
Control.....	6±1.6	5.81±.116	3.93±.123	.12±0.043
Early deflorated.....	10±2.0	5.97±.104	3.95±.152	.11±.037
Third period (from flowers of Aug. 23 to Sept. 15):				
1 boll per branch.....	6±1.6	6.32±.107	2.68±.123	.09±.053
Control.....	0±0	5.79±.084	2.77±.138	.52±.090
Early deflorated.....	4±1.4	5.63±.111	2.84±.141	1.34±.196

<sup>a</sup> 90 bolls.

<sup>b</sup> 25 bolls.



There are certain statistically significant variations in the number of seeds per lock at different periods in the different treatments. These effects, however, are in opposite directions in the two varieties. The control Acala cotton appears to have had more seeds per lock than the 1-boll-per-branch Acala in each of the three periods, but in Pima the relationship is reversed. Conversely, a significantly smaller number of aborted ovules, or motes, per boll occurred in the control Acala bolls during the second period than in the 1-boll-per-branch bolls or in the early-deflorated bolls. In the Pima variety the number of motes per boll for the second period was significantly greater in the control and early-deflorated plants than in the 1-boll-per-branch plants.

Imperfect seeds were not sufficiently abundant in Acala seed cotton of the first period to appear noteworthy. The number of these in the cotton of the second period, however, attracted attention, and they were counted. Three, four, and ten per cent of the second-period seeds were found to be imperfect in the 1-boll-per-branch, control, and early-deflorated cotton, respectively. Imperfect seeds are smaller than normal seeds, and the fiber attached to them is short and wasty. Fiber of this character may be a contributing factor to the neps found after ginning and adds to the quantity of waste to be taken from the cotton by the mills. Less than one-fourth of the cotton from the early-deflorated plants, however, was produced from the second-period flowers.

The second-period early-deflorated Acala cotton, as may be noted in Figure 3, contained some foreign material in the form of pieces of seed coats torn from the imperfect seeds in ginning. The cotton from the control plants contained fewer neps than that from the early-deflorated plants, but in no respect other than in length was the control cotton of as high a quality as the fiber from the 1-boll-per-branch plants.

TABLE 5.—Mean weight per seed of three 25-gm. samples each of Acala and Pima seed, from flowers of each period of season (July 1 to September 15, 1928), for each of three treatments

Period and treatment	Acala				Pima			
	Lot No. 1	Lot No. 2	Lot No. 3	Mean	Lot No. 1	Lot No. 2	Lot No. 3	Mean
First period (from flowers of July 1 to Aug. 4):	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>
1 boll per branch.....	0.149	0.150	0.152	0.150	0.137	0.138	0.133	0.135
Control.....	.135	.137	.134	.135	.124	.123	.124	.124
Early deflorated.....	.132	.133	.132	.132	.130	.129	.126	.128
Second period (from flowers of Aug. 5 to 22):								
1 boll per branch.....	.142	.142	.141	.142	.135	.136	.136	.136
Control.....	.127	.123	.124	.125	.120	.117	.115	.117
Early deflorated.....	.124	.123	.118	.122	.126	.124	.123	.124
Third period (from flowers of Aug. 23 to Sept. 15):								
1 boll per branch.....	.142	.146	.137	.142	.135	.137	.133	.135
Control.....	.130	.133	.130	.131	.115	.113	.111	.113
Early deflorated.....					.110	.114	.114	.113

#### MEAN WEIGHT OF SEEDS

In both the Acala and Pima varieties the heaviest seeds were produced by the 1-boll-per-branch plants in each of the three periods (Table 5); the differences for the means of triplicate 25-gm. samples

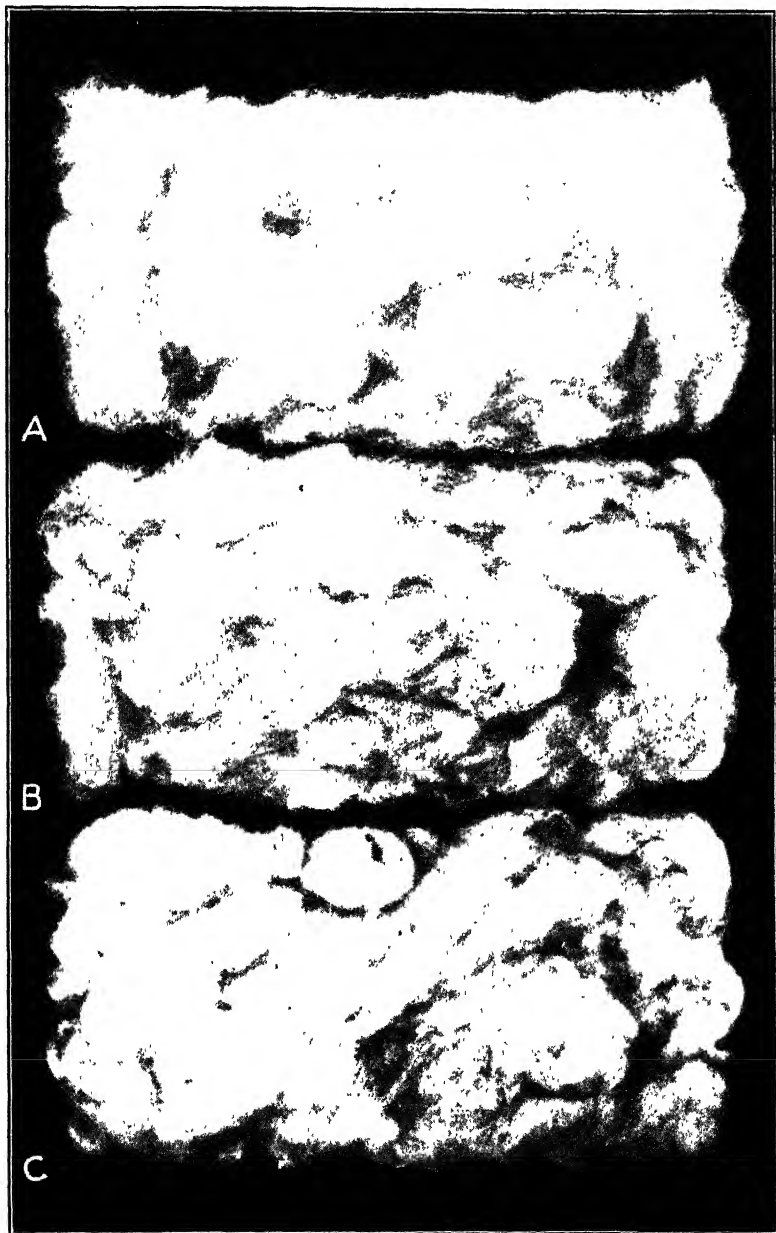


FIGURE 3.—Roller-ginned *Acacia* cotton from flowers of second period. A, One-boll-per-branch cotton. This was superior to either B or C. B, Control. C, Early-defoliated cotton. Foreign material in the form of pieces of seed coats torn from imperfect seed in ginning is to be noted in this sample. It contained the greatest number of neps. Significant differences were not noted between the control and the early-defoliated cotton of the first period, which cotton constituted the bulk of the crop in each case

varied from 8 to 19 per cent. Seeds from the control Acala plants in nearly all cases were heavier than those from the early-defoliated plants, but the differences were not marked. Seeds from the early-defoliated Pima plants were heavier than seeds from the control plants except in the last period, where no difference in average weights was found.

In both varieties the seed weight appears to decrease as the boll load on the plants increases with respect to plant size.

#### SEED FUZZINESS

Kearney and Harrison<sup>6</sup> have reported that, in Arizona, seeds in bolls produced on higher fruiting branches and at fruiting-branch nodes farther removed from the main axis of Pima Egyptian cotton plants have less fuzz on them than have seeds on lower branches and on nodes near the main axis of the plant. Seeds produced under the different treatments herein described were examined for variations in this character, but no evidence was found that the differences in the nutritional relationships of these plants caused marked differences in seed fuzziness.

#### LENGTH OF FIBER

The length of both Acala and Pima fiber (Table 6) produced from flowers of the second period was longer than that from flowers of the first period. The treatments did not influence this character during the first period, and no material difference was found in the second period. The difference in length between the Acala fiber from the 1-boll-per-branch plants and that from the early-defoliated plants of the second period is  $0.4 \pm 0.10$  grade,<sup>7</sup> which is significant, the fiber from the 1-boll-per-branch plants being the longer; but no difference was found between the 1-boll-per-branch plants and the control plants. The results obtained indicate that the number of bolls carried by these plants did not influence the length of fiber to the extent that it did other characters previously discussed.

TABLE 6.—Relative mean length<sup>a</sup> of unginned fiber in Acala and Pima cotton, from flowers of each period of season (July 1 to September 15, 1928), for each of three treatments

[Values represent mean length of fibers attached to 20 seeds, each seed from a different boll selected at random from 100-boll samples]

Period and treatment	Acala	Pima
First period (from flowers of July 1 to August 4):	Grade	Grade
1 boll per branch.....	1.0±0.07	7.9±0.07
Control.....	3.9±.04	8.0±.05
Early defoliated.....	3.9±.07	8.1±.06
Second period (from flowers of August 5 to 22):		
1 boll per branch.....	4.1±.07	8.9±.06
Control.....	4.4±.13	8.7±.05
Early defoliated.....	4.0±.07	8.6±.04
Third period (from flowers of August 23 to September 15):		
1 boll per branch.....		7.2±.03
Control.....		7.2±.03
Early defoliated.....		7.1±.08

<sup>a</sup>A grade difference of 1 equals one-eighth inch. The grade 4, for example, corresponds to combed fiber ends  $1\frac{1}{4}$  inches from center line of seed.

<sup>6</sup> KEARNEY, T. H., and HARRISON, G. J. VARIATIONS IN SEED FUZZINESS ON INDIVIDUAL PLANTS OF PIMA COTTON IN ARIZONA. Jour. Agr. Research 37: 465-472, illus. 1928.

<sup>7</sup>The length of fiber was measured by the method of "grade" as devised by Kearney: KEARNEY, T. H. SEGREGATION AND CORRELATION OF CHARACTERS IN AN UPLAND-EGYPTIAN COTTON HYBRID. U. S. Dept. Agr. Dept. Bul. 1164, 58 p., illus. 1923.

## BOLL-MATURATION PERIODS

Information as to the influence of the plant load on the maturation period of the bolls was available from the dated tags placed on each flower during the period from August 5 to August 22, inclusive. These data have been tabulated only for a comparison between the 1-boll-per-branch plants and the early-deflorated plants. The results of this comparison are given in Table 7.

TABLE 7.—Mean date of flowering and mean days from flower to open boll for 1-boll-per-branch and early-deflorated Acala and Pima plants from August 5 to August 22, inclusive, 1928

Variety and treatment	Mean date of flowering from August 5 to 22	Mean days from flower to open boll
Acala:		
1 boll per branch.....	Aug. 13 <sup>a</sup> (13.12).....	48.3±0.25
Early deflorated.....	Aug. 11 <sup>a</sup> (11.63).....	46.5±.15
Difference in boll-maturation periods.....		+1.8±.29
Pima		
1 boll per branch.....	Aug. 14 <sup>a</sup> (14.20).....	55.6±.13
Early deflorated.....	Aug. 12 <sup>a</sup> (12.70).....	57.5±.08
Difference in boll-maturation periods.....		-1.9±.15

<sup>a</sup> Fractional date of flowering.

The bolls on the 1-boll-per-branch Acala plants required  $1.8 \pm 0.29$  days longer to mature than did those on the early-deflorated plants. In the Pima variety the relationship is found to be reversed.

These relatively very small differences in length of the boll-maturation periods are considered especially noteworthy in view of the marked effect of seasonal factors upon maturation periods. Pima bolls set in the latter part of the season may require 20 days longer to mature than those set in July.

#### SUPPLEMENTARY EXPERIMENT TO DETERMINE EFFECT OF MODIFIED EARLY DEFLORATION

In view of the increased yields anticipated as a result of the early defloration of the Acala plants, it seemed desirable that the experiment herein described should be supplemented by a minor experiment in which the early-defloration method was modified for the purpose of securing information as to its possible commercial practicability. Accordingly, on July 9, all bolls, flowers, and squares of considerable size were stripped from alternate plants in an outside row of the same plot in which the main experiment was conducted. The stripped plants again produced open flowers on July 23. As is shown in Table 8, the effect of defruiting and bud removal upon yields in this experiment was similar to that in the more carefully conducted experiment in which the flowers and large squares were systematically removed during the early flowering period. The results indicate a possible increase in yield of more than 25 per cent.

TABLE 8.—Amount of seed cotton produced by 100 Acala plants from which all bolls, flowers, and large squares had been stripped on July 9, and by alternately spaced control plants

Treatment	Seed cotton produced		
	First picking (Oct. 10)	Second picking (Jan. 3)	Total for season
Stripped	Pounds 26.3	Pounds 4.4	Pounds 30.7
Control	Pounds 19.0	Pounds 4.7	Pounds 23.7

## CLASSIFICATION OF SAMPLES BY BUREAU OF AGRICULTURAL ECONOMICS

After the field-laboratory examinations had been completed, samples of the ginned cotton from the second period of the main experiment with Acala and Pima and from both pickings of the supplementary experiment with Acala were forwarded to the Bureau of Agricultural Economics for independent classification. The results of this classification are given in Table 9.

TABLE 9.—Classification of cotton samples by Bureau of Agricultural Economics <sup>a</sup>

Experiment, variety, and treatment	Grade <sup>b</sup>	Length of staple <sup>c</sup>	Fiber (body)	Strength	Remarks
Major experiment, second period, roller ginned:					
Acala—		Inches			
1 boll per branch	GM E. W. <sup>d</sup>	1 $\frac{3}{32}$	Medium	Fair	Fairly free of neps.
Control	do	1 $\frac{3}{32}$	do	do	Do.
Early deflorated	SM E. W.	1 $\frac{1}{16}$	do	do	Slightly neppy.
Pima—					
1 boll per branch	No $\frac{1}{4}$ A. E. <sup>e</sup>	1 $\frac{1}{8}$	Fine	Strong	Good character.
Control	N. $\frac{1}{4}$ A. E.	1 $\frac{5}{8}$	do	Normal	Slightly neppy.
Early deflorated	do	1 $\frac{1}{2}$	do	Strong	Good character.
Supplementary experiment, saw ginned:					
Picked Oct. 10 (three-fourths of crop)—					
Acala—					
Control	SM E. W.	1 $\frac{1}{32}$	Medium	Normal	Neppy.
Early bolls and buds stripped	do	1 $\frac{1}{32}$	do	do	Less neps than control.
Picked Jan. 3 (one-fourth of crop)—					
Acala:					
Control	do	1 $\frac{1}{16}$	do	do	Neppy.
Early bolls and buds stripped	do	1 $\frac{1}{16}$	do	do	Neppy, equal to control.

<sup>a</sup> Under the direction of A. W. Palmer.

<sup>b</sup> GM E. W. is Good Middling Extra White, SM E. W. is Strict Middling Extra White.

<sup>c</sup> Average uniformity of length was regular in all cases.

<sup>d</sup> Extra-white standards.

<sup>e</sup> American-Egyptian standards.

The cotton classes differentiated between the early-deflorated and the control Acala from the second period with respect to grade, length of staple, and number of neps. The most outstanding difference was expected from the cotton of the second period, but this cotton constituted only a small portion of the entire crop. It will be noted that the early-deflorated Acala plants, which carried the heaviest loads of bolls, produced  $\frac{1}{32}$ -inch shorter lint during the

second period than the control or the 1-boll-per-branch plants of this variety, the difference being nearly the same as that indicated by the method of grade in Table 6. The early-deflorated Pima plants, while not more heavily laden in proportion to their size, nevertheless produced 24 per cent more cotton than the control plants, and the cotton classers' report shows that the early-deflorated cotton was of higher quality than the cotton produced by the control plants.

The cotton classers differentiated between the samples from the supplementary experiment with Acala only in the number of neps. Confirming observations of the writer and of others who examined the samples, the cotton classers found that the cotton from the control plants contained more neps than that from plants whose yields had been increased by more than 25 per cent by the removal of early bolls and squares. The latter result indicates that early defloration, or stripping, as a method of increasing yields is warranted from the standpoint of the quality of the fiber produced.

#### SUMMARY AND CONCLUSIONS

An experiment was conducted with the Acala upland and the Pima Egyptian varieties of cotton, for the purpose of determining (1) whether yields could be increased by delaying for several weeks the initiation of flowering and boll setting, and (2) the effect of both an increased and a decreased number of bolls per plant upon the characteristics of the bolls and fiber produced.

The modifications in the number of bolls per plant were brought about in two ways: (1) By allowing a boll to develop only at the first node of any fruiting branch, and (2) by removing all flowers during the first 25 days of the flowering period. The results of these treatments were checked against the number of bolls produced by untreated control plants. The resultant yields in number of bolls per plant were: For Acala, 10.7 and 20.1 from 1-boll-per-branch and early-deflorated plants, respectively, as against 16.8 from untreated plants; and for Pima, 16.6 and 49.9 from 1-boll-per-branch and early-deflorated plants, respectively, as against 42.4 from untreated plants. While the effect of the first treatment was to decrease the number of bolls, substantially increased yields resulted from the second treatment (early defloration) in both varieties, irrespective of whether the yields were measured in terms of the number of bolls, the weight of the seed cotton, or the weight of the fiber produced.

An increase of more than 24 per cent in the yield of Acala cotton was obtained in a supplementary experiment in which all bolls and flowers and the larger squares were stripped from 100 plants alternately spaced with control plants. The plants were treated in this manner on the eighth day of the flowering period, and they again produced flowers 14 days later. The method used in this experiment may prove practicable commercially, and the increased yields obtained from it indicate that it might be profitable.

An inverse relation was found, in both Acala and Pima, between the number of bolls per plant and the mean weights of the open bolls and burs and of seed cotton per boll and seed per boll (also Pima fiber per boll) for the bolls resulting from the second-period flowers (August 5 to 22). It was not until the latter part of the summer that the early-deflorated Pima plants had set a greater number of

bolls than the control plants, and, in the characters mentioned above, the early-deflorated Pima plants were intermediate between the 1-boll-per-branch and the control plants. In general the weight of the burs was influenced by the treatments to a greater extent than the weight of the seed cotton, and the weight of the seed more than the weight of the fiber.

The treatments did not result in significant differences in the number of locks per boll, nor did they materially influence boll-maturation period.

The classification of the Acala cotton from the second-period flowers brought out differences which favored the fiber from the 1-boll-per-branch and the control plants. In samples representing both pickings from the supplementary Acala experiment, the only differences were in the number of neps; fewer neps were found in the samples from plants stripped of early bolls and buds.

The cotton from Pima 1-boll-per-branch plants received the highest ratings, that from the early-deflorated plants being intermediate and that from the control plants lowest.

It has been observed that the yields of determinate strains of cotton are lower than the yields of strains that ordinarily abort a considerable number of their early buds and bolls. The Acala strain used in experiments herein reported has proved to be less determinate in its growth and better suited to Arizona conditions than other strains with which it has been compared.

The marked increase in yields obtained by early defloration is particularly noteworthy in view of the fact that similar results were obtained with both the upland Acala and the Pima Egyptian varieties, which are very different, not only in their morphological but also in many of their physiological characteristics.

# SOME NEMIC PARASITES (OXYURIDAE) OF COLEOPTEROUS LARVAE<sup>1</sup>

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## INTRODUCTION

No group of nemas is found in a greater variety of hosts than the Oxyuridae. Representatives occur in every class of vertebrates and in addition are common parasites of vast numbers of invertebrates.

Early investigators of this group, inspired by an interest almost purely scientific, studied and described a considerable number of not very closely related species from both vertebrate and invertebrate hosts, placing them in the genus *Oxyuris*. Subsequent workers, influenced by an increased emphasis placed on the economic phases of helminthology, devoted their efforts largely to parasites infesting vertebrates, especially domesticated animals and man. As a result of this intensified study, the genus *Oxyuris*, as originally conceived, has been broken up into about 60 genera. But until recently the nemic parasites occurring in invertebrates have received scant attention, and many of the early described species are still carried in the literature, including various textbooks, as belonging to the genus *Oxyuris*, although in the modern conception of the genus this is obviously incorrect.

During the last decade the ever-increasing appreciation of the value of parasites in controlling injurious insects has caused nematologists to realize that the nemic parasites of invertebrates may be of more than purely scientific importance and to view them with renewed interest. The probable economic rôle of some of these forms, including several of the oxyurids, has already been demonstrated. Among them may be mentioned *Neoaplectana glaseri* Steiner (8),<sup>3</sup> infesting the larvae of the Japanese beetle, *Popillia japonica* Newman.

These oxyurids of invertebrates present many difficulties to one who would contribute to the knowledge of their taxonomy. In the first place, taking the group as a whole, the characters that have been most used, not only as a basis for separation into major groups but also as a means of identifying genera and species, are largely characters of the male. Among nemas infesting insects are many species the males of which are either rarely seen or unknown, and in those cases where males are found there is often a pronounced sexual dimorphism. This dimorphism involves not only size and sexual characters but many other characters as well. For example, females may have conspicuous papillae on the head, whereas on males of the same species these papillae are either absent or so reduced as to be indis-

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<sup>2</sup> The writer is indebted to Gertrude L. Power, formerly of the Office of Nematology, Bureau of Plant Industry, U. S. Department of Agriculture, for assistance in conducting the investigations and in preparing the drawings and manuscript; to A. G. Böving, Bureau of Entomology, U. S. Department of Agriculture, for identification of the host insects; and to Frank Blanchard, University of Michigan, for collecting and sending the insects from which a large number of the parasites were obtained.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 482.



tinguishable. The shape of the head, the character of the pharynx, the shape of the esophagus, and many other structures may show considerable variation between the two sexes. Hence, in cases where two or more species inhabit the same insect host, the matching of males and females may become an exceedingly perplexing problem.

Investigation of marine and many other free-living species has shown that a female containing mature ova is nearly full grown, so that corresponding measurements of a number of such females of the same species will be fairly uniform. This is less likely to be true of the oxyurids of insects. In some species a female may nearly double in size (by increasing in both length and breadth) after she has begun depositing eggs. The growth of such females has already been noted by Bütschli (7), Steiner (7), and others. To some extent a corresponding growth seems to occur in sexually mature males. Another cause of variation in measurements is found in the ability of the parasites to elongate or shorten the anterior or neck region of the body. In the living specimen the length of the neck and the relative position of such structures as the nerve ring and the excretory pore are not necessarily constant, and in fixed material the variation may be even more noticeable. This subject is mentioned, not with a view to belittling the value of measurements, but in order to point out certain facts that must be given consideration in comparing individuals.

In the following formulas the writer has not attempted to give the limits of variation, but has taken measurements from the largest specimens available.

#### TERMS

**AMPHIDS.**—This term is used here to include all structures, both internal and external, which go to make up these sense organs. The appearance of the amphids at the points where they open to the exterior, as seen when the head is viewed from the front, is referred to as their external manifestation. This does not always indicate a purely surface demarcation, but may result partly from an enlargement of the tube leading backward, which, lying just below the cuticle, may be seen through it. This enlargement is referred to as the vestibule.

**ISTHMUS.**—That part of the esophagus between the anterior portion and the cardiac bulb, often set off from the former by a distinct constriction, is referred to as the isthmus, a term which has already been used with this meaning. The isthmus is, strictly speaking, the anterior neck of the cardiac bulb.

**MYOLABIA.**—Cobb (2) has recently expressed the opinion that the eight cephalic elevations occurring on the head of the female of *Thelastoma attenuatum* Leidy are the external manifestations of the muscle fields and has proposed for them the name myolabia. After studying a considerable number of species of this and related genera, the writer is not inclined to regard the elevations as lips. On the head of the female of *Hystriognathus rigidus* Leidy, corresponding exactly to the myolabia of *T. attenuatum*, are eight elevations which the writer believes any nematologist would unhesitatingly designate as papillae. Only slightly less papilloid in character are the innervated cephalic elevations on the head of the female of *T. papilliferum*, n. sp. On the female of *T. macramphidum*, n. sp., one finds similarly placed elevations, more or less papilloid but without apparent nerve endings. In the female of *Blatticola blatticola* (Gale) these structures are broad,

considerably flattened, and more or less incurved, a condition likewise found in the female of *Aorurus subcloatus*, n. sp., although in the latter the elevations are subrectangular in outline when seen from the front. In *Scarabanema cylindricum*, n. sp., these structures are not sufficiently elevated to break noticeably the even contour of the head, but when seen from the front their margins form a faint scroll-like pattern around the mouth. The structures in *Binema ornata* Travassos and *B. binema* Travassos are large and considerably elevated, similar to but somewhat larger than those of *T. attenuatum*, and they assume a truly labiumlike character. The head of the female of *T. robustum* Leidy is more or less intermediate between the extreme condition found in *T. myolabiatum* Cobb and the more common condition found in such forms as *Binema binema*. The head of *T. robustum*, when viewed from the front, is distinctly 8-lobed, but the lobes are not so deeply cut nor do they extend toward the mouth as in *T. myolabiatum*. But the head of *T. robustum* also bears the eight smaller lobelike elevations pointing inward toward the mouth and somewhat resembling those of *Blatticola blatticola*. To which of the two subgenera of *Thelastoma* proposed by Cobb such a species as *T. robustum* should belong is problematical.

Failure to see nerve endings in these cephalic elevations merely means that they are not conspicuously innervated. It is the writer's opinion that throughout the series (with the possible exception of *Thelastoma myolabiatum*) these structures are homologous and that ontogenetically they are papillae. Myolabia may be an appropriate term for the structures as found on the heads of *T. myolabiatum*, *T. robustum*, *Binema binema*, and others, where they are more or less labial in character. To apply it to the distinctly papilloid structures of *Hystriognathus rigidus* and *T. papilliferum* would, it seems, lead to confusion. At what point in the series they cease to be papillae and become myolabia is another matter, and one which at present is not altogether clear.

The posterior end of the alimentary tract (see fig. 3, A) of many scarabaeid larvae is pronouncedly enlarged, and the food material contained therein gives the posterior part of the animal its characteristic dark appearance. This region of the intestine has been referred to by some German helminthologists as the "Blinddarm," although it is not, strictly speaking, a caecum, but rather a greatly dilated convolution in the posterior part of the intestine. This intestinal enlargement is the habitat of several nemic species, including those dealt with in this paper.

#### TAXONOMY

The information furnished in the following keys should serve as a brief diagnosis by which the related genera and species may be differentiated. An attempt has been made to include in the key to the genera a sufficient number of characters to differentiate them, not only from one another but from related genera. At present the genus *Thelastoma* is in a very confused condition. It contains a considerable number of species, many of which have been inadequately studied and described, and some of which obviously do not belong to the genus. Until the whole group has been carefully worked over, it is impossible to key the genus or provide any given species with a specific diagnosis that will positively differentiate it from all other species of the genus.

## KEY TO THE GENERA

1. Female with distinct, subspherical median bulblike enlargement of the esophagus.....AORURUS  
 Female without distinct median bulblike enlargement of the esophagus. Cephalic elevations (papillae or myolabia?), when present, eight in number, never four; ovaries two. Male with spicule.....2
2. Total length of female, up to 5 mm.; slender, pointed caudal spike; external amphids elliptical, comparatively large; anus of male strongly salient; papillae grouped close to anus except for pair on caudal spike; excretory pore (both sexes) near cardiac bulb.....THELASTOMA  
 Total length of female, up to 8 mm.; short, conical, blunt posterior terminus. External amphids very small, nearly circular; anus of male not strongly salient; most anterior pair of papillae somewhat removed from anus; excretory pore (both sexes) posterior to cardiac bulb by a distance greater than the corresponding body diameter.....SCARABANEMA

## KEY TO THE SPECIES OF THELASTOMA DESCRIBED IN THIS PAPER

1. Vulva distinctly salient; external amphids (both sexes) very large; female head with four broad, lobelike elevations without apparent innervation.....*T. macramphidum*  
 Vulva not distinctly salient; external amphids (both sexes) of moderate size; female head with four distinctly papilloid, innervated elevations.....*T. papilliferum*

## KEY TO THE KNOWN SPECIES OF AORURUS

1. Vulva near anus.....*A. subcloatus*  
 Vulva not near anus, nearer middle of body.....2
2. Isthmus twice as long as broad; neck region elongated; annules very wide; parasites of Blattidae.....*A. diesingii*  
 Isthmus as wide as or wider than long; neck region not elongated, annules of moderate width; parasites of Myriopoda.....*A. agile*

## KEY TO THE KNOWN SPECIES OF SCARABANEMA

1. With three conspicuous, yellowish bodies near the vagina.....*S. leuckarti*  
 Without conspicuous bodies near vagina.....2
2. Ova much elongated, 89 $\mu$  long by 26 $\mu$  in diameter.....*S. brevicaudatum*  
 Ova less elongated, 70 $\mu$  long by 45 $\mu$  in diameter.....*S. cylindricum*

## FAMILY OXYURIDAE

## GENUS SCARABANEMA, N. G.

*Scarabanema*, n. g.

## GENERIC DIAGNOSIS:

*Female*.—Body comparatively long, nearly cylindrical, its maximum diameter somewhat anterior to the middle. Caudal spike conical, short. Pharynx very short, sometimes armed with three teeth. Anterior portion of esophagus nearly cylindrical, contracted where it joins isthmus, the latter short but distinct, with a diameter distinctly less than the anterior portion of the esophagus. Cardiac bulb nearly spherical. Excretory pore posterior to cardiac bulb. Excretory ducts very large and conspicuous. Ova, as compared with size of body, small (in known species 70 $\mu$  to 100 $\mu$  long by 25 $\mu$  to 70 $\mu$  broad), numerous (in *S. cylindricum* 800 to 1,000), not distinctly flattened on one side. Reproductive system double, vulva near middle of body.

*Male*.—Much smaller than female. Tail ending in slender caudal spike; anus but slightly salient. A pair of papillae (one right and one left) somewhat anterior to the anus, probably a pair of weakly developed papillae opposite the anus, a single median duplex papilla slightly posterior to the anus, and a pair of papillae on the base of the caudal spike. Excretory pore posterior to cardiac bulb,

Parasites of the posterior end of the intestine of coleopterous larvae of the family Scarabacidae.

Type species.—*Scarabanema cylindricum*, n. sp.

*Scarabanema cylindricum*, n. sp. (Figs. 1 to 5, inc.)

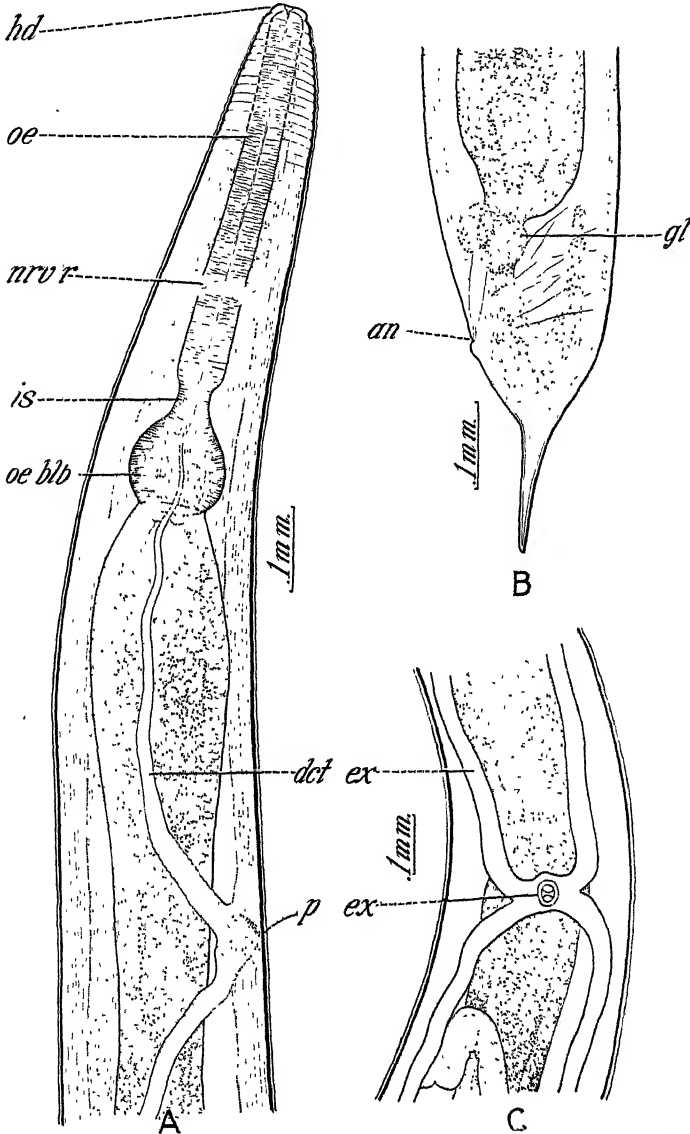


FIGURE 1.—*Scarabanema cylindricum*, ♀. A, Anterior end, lateral view; *hd*, head; *oe*, esophagus; *nrv r*, nerve ring; *is*, isthmus; *oe blb*, esophageal bulb; *dct ex*, excretory duct; *p ex*, excretory pore; B, posterior end, lateral view; *gl*, gland; *an*, anus; C, excretory pore and ducts, ventral view; *dct ex*, excretory duct; *p ex*, excretory pore

Female:

5.4	10.	'52'	96.	6 mm.
2.4	3.4	5.4	2.4	

The body is nearly cylindrical, 3.5 to 6 mm. long. The cuticle is traversed by coarse, transverse striae from  $6\mu$  to  $10\mu$  apart. On the posterior portion of the body, at varying intervals, usually from 0.1 mm. to 0.2 mm. apart, a transverse stria takes the form of a rather pronounced groove. Alae are not present. The head is subtruncate, with a constriction setting off a head region (fig. 1, A),  $10\mu$  long. The first annule back of the head region is  $15\mu$  to  $18\mu$  wide. The anterior 10 to 12 annules are more conspicuous than those elsewhere on the body, giving the anterior part of the conoid neck region a crenate contour. When seen from the front the subtriangular mouth is without lips and is surrounded by eight rather faint, more or less incurved, very slightly elevated, lobelike structures (fig. 2, B), not distinctly papilloid in appearance and not observed to be innervated. The rather conspicuous, small, faintly elliptical, amphidial markings (fig. 2, B) occupy the usual positions on the anterior outer margin of the head. The simple, very short, conical pharynx (fig. 2, C) is armed at the base with three structures which, when the head is seen in profile, appear as sharp, inward-pointing and forward-pointing teeth, but when viewed from in front (fig. 2, B) have the appearance of broad, triangular plates. The esophagus enlarges very slightly

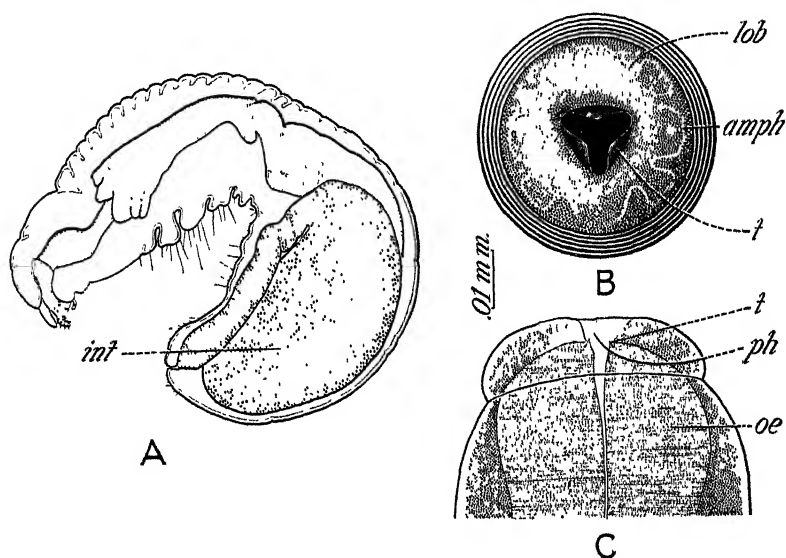


FIGURE 2.—A, Digestive tract in typical scarabaeid larva; *int*, intestine; B, *Searabaneia cylindricum*, ♀, end view of head; *lob*, lobe; *amph*, amphid; *t*, tooth; C, *Searabaneia cylindricum*, ♀, dorsal view of head, *t*, tooth; *ph*, pharynx; *oe*, esophagus

where it joins the pharynx. The anterior portion (fig. 1, A) is nearly cylindrical, increasing slightly and gradually in diameter posteriad. There is an abrupt reduction in size where it joins the isthmus, the latter having a diameter two-thirds as great as the adjacent end of the anterior portion. The cardiac bulb (fig. 1, A, *oe blb*) is broadly pyriform, and has a diameter equal to about four-sevenths of the corresponding body diameter. It possesses the valvular apparatus characteristic of the group, but the fact is somewhat obscured by the density of the bulb.

The ventriculus varies in different individuals. In some cases the enlargement is rather gradual, whereas in old females the dilation is slightly telescoped over the bulb. Behind the ventriculus the intestine is two-fifths as wide as the corresponding part of the body except at the posterior end, where there is another slight dilation. The more or less straight, conical rectum has a length about equal to the body diameter at the anus. At its junction with the intestine it is surrounded by the usual rectal glands (fig. 1, B), probably three in number. In some specimens the body tapers more or less evenly from the anus to the point of the tail, producing a broad, conical terminus; in others, probably older females, it rounds off back of the anus and is then drawn out into a short, blunt terminal spike, about 0.25 mm. long from the anus.

The excretory system (as in all species here described) is of the H-shaped type. A right and a left posterior branch, contained in the lateral cords, extend down toward the ventral surface as they approach the

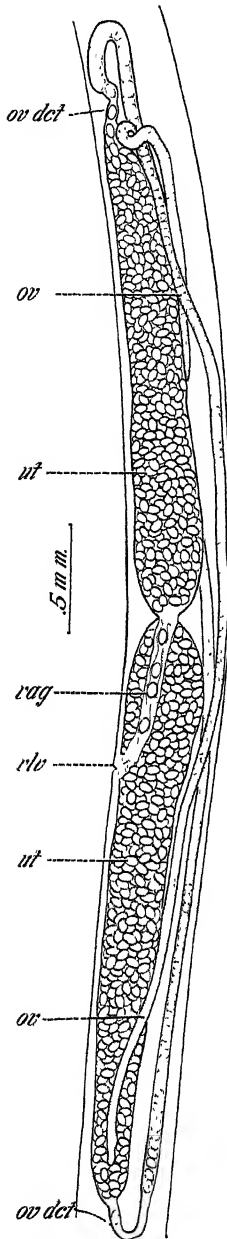


FIGURE 3.—*Scarabanema cylindricum*, ♀, reproductive system, lateral view; *ov dcl*, oviduct; *ov*, ovary; *ut*, uterus; *vag*, vagina; *rlv*, vulva

excretory pore. Here they are joined by two similar anterior branches, all four ducts uniting near the excretory pore, which lies 0.5 mm. back of the cardiac bulb. (Fig. 1, C.) The ducts are very large and conspicuous in the region of the pore (fig. 1, A and C), but distally they become smaller. The nerve ring encircles the esophagus about three-fourths the distance from the head of the isthmus.

The reproductive system is double. The vulva is near the middle of the body and is not salient. The tubular vagina (fig. 3) has a length slightly greater than the corresponding body diameter, and passes antieriad to join the uteri. Usually, in this group of oxyurids, the proximal end of the vagina is differentiated into a region with thicker and more muscular walls and a narrower and sometimes more heavily cuticularized lumen. In this species, however, a differentiation of this character is not very conspicuous, although the walls of the vagina close to the vulva seem slightly more muscular than elsewhere. The general structure and position of the female gonads can best be understood by referring to Figure 3. The ova are ellipsoidal ( $70\mu$  by  $45\mu$ ) and are deposited before segmentation.

#### Male:

9.1 17. 'M 93.  
3.3 4. 4.7 2. 1.15 mm.

Body moderately slender, total length 1.15 mm., diameter at middle of body  $54\mu$ , caudal spike  $80\mu$  long from anus. Cuticle faintly and rather finely striated. In the neck region the annules are  $1.5\mu$  to  $2\mu$  in width, increasing to about  $3\mu$  in the middle of the body. The head is subtruncate, and a wide, almost imperceptible constriction sets off an exceedingly short head region. The pharynx is so short as to be nearly nonexistent. The anterior portion of the esophagus (fig. 4, *oe*) is nearly cylindrical. It is faintly expanded at its anterior end, where its diameter ( $12\mu$ ) nearly equals that of the head region. It is slightly and gradually expanded at the middle, where its diameter ( $15\mu$ ) is

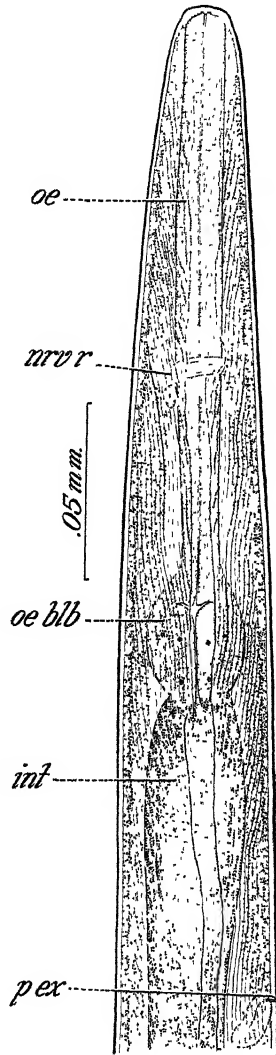


FIGURE 4.—*Scarabanema cylindricum*, ♂, anterior end, lateral view; *oe*, esophagus; *nrr*, nerve ring; *oe blb*, esophageal bulb; *int*, intestine; *per*, excretory pore

about half the corresponding body diameter. It gradually contracts in front of the cardiac bulb (fig. 4, *oe, blb*) to a diameter of  $11\mu$ , but no distinct isthmus is differentiated. The cardiac bulb is slightly elongated with a diameter of  $29\mu$  and a length of about  $34\mu$ . The small but rather distinct valvular apparatus is located well forward. The bulb contains at least nine nuclei, probably of the muscle cells. The intestine (fig. 4) is slightly expanded at its anterior end, the wall in this region being comparatively thin and the lumen correspondingly enlarged, thus forming a rather distinct ventriculus. The rectum has a length ( $55\mu$ ) slightly more than twice the body diameter at the anus ( $23\mu$ ). Exactly what part of this is cloaca was not determined. In the specimen studied the rectum was

considerably expanded in the middle. The anus is but slightly salient. The testis is reflexed.

The spicule (fig. 5, B) is  $43\mu$  long, not expanded at the proximal end, and ends distally in a sharp point. It is conspicuously expanded in the middle to a diameter of  $5\mu$ , the expansion forming a backward-pointing barb on the antero-ventral side.

A pair (one right and one left) of preanal papillae (fig. 5, A and B) are located  $23\mu$  in front of the anus, each being about  $4\mu$  high by  $6\mu$  broad at the base. A pair of papillae, one on each side of the anus, are evidently present, although they do not stand out conspicuously. Slightly posterior to the anus ( $5\mu$ ) there is a median, duplex papilla with two distinct nerve endings, and there are a pair of papillae on the caudal spike about  $25\mu$  back of the anus. In lateral view there appears to be a small, narrow depression immediately in front of the median postanal papilla, covered over with the cuticle; but this could not be seen in ventral view.

*Host*.—Scarabaeid larvae belonging either to the Melolonthinae or the Rutelinae. Four of these larvae (from three of which *Scarabanema cylindricum*

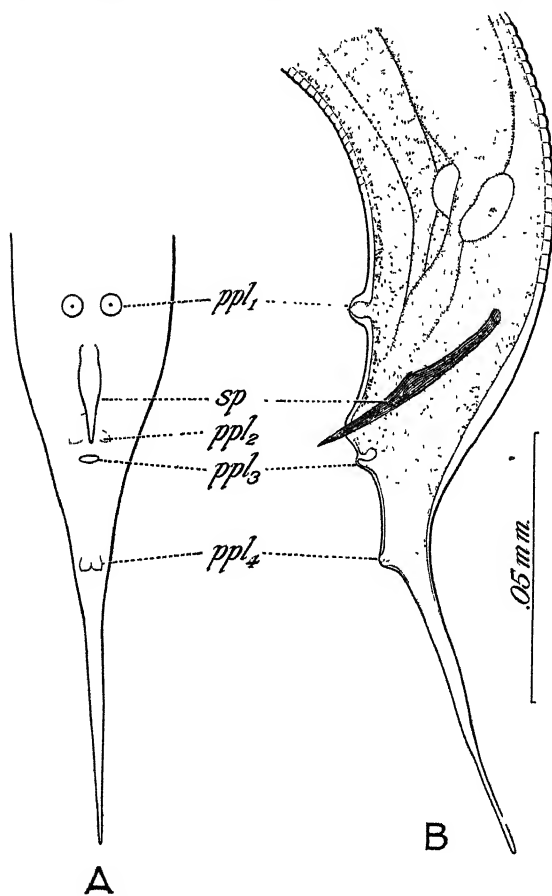


FIGURE 5.—*Scarabanema cylindricum*, ♂: A, Posterior end, ventral view; B, posterior end, lateral view; *sp*, spicule, *ppl1*, preanal papillae; *ppl2*, papillae, one on each side of anus, *ppl3*, median, duplex, postanal papilla; *ppl4*, papillae on caudal spike

had been removed) were submitted to A. G. Böving<sup>3</sup> of the Bureau of Entomology, United States Department of Agriculture, who reported as follows:

"The four larvae are all the same species, and according to all the characters, with the exception of two, this species belongs to the subfamily Rutelinae.

"Like the rest of the known Rutelinae larvae, it possesses 4-jointed antennae, each of the abnormal segments one to six having three dorsal transversal bulges, densely beset with small thorns; the legs have one distinct claw; below the anus are two almost parallel longitudinal rows of pointed spines and the anal slit forms a simple transverse line. The latter character is very important as it distinctly separates the Rutelinae from the Melolonthinae which have a Y-shaped

anus. Unfortunately only one of the submitted four specimens shows this region intact [in the others it was destroyed during the removal of the parasites], but in this one, the shape of the anus is very evident. The two extraordinary characters referred to above are (1) the presence of 16 to 17 stridulating teeth on the dorsal (buccal) margin of the maxillae—all other Rutelinae have only from 6 to 10—and (2) no stridulating area on the ventral (buccal) side of the mandibles, while all other Rutelinae larvae have a very distinct mandibular stridulating area. The end of each of the mandibles is oblique and without many pointed teeth, just as normal in all Rutelinae as in the Melolonthinae.

"By the deviating maxillar and mandibular stridulating structure the submitted larval form is easily recognized, but in the museum collection of the Rutelinae larvae none of the genera or species represented possesses it, and as far as I know nowhere in the literature is a similar larva described."

*Location*.—Posterior end of intestine.

*Locality*.—Woods Hole, Mass., U. S. A.

*Type specimens*.—United States National Museum Helminthological Collection No. 8108.

*Paratypes*.—No. 8109.

*Technic*.—Male specimen killed in hot 60 per cent alcohol, containing 3 per cent glycerin from which the alcohol was evaporated. It was mounted and studied in glycerin jelly. This method of treatment may have resulted in a slight increase in length. Females were studied while living and after various treatments. Formula from specimens stilled with osmic vapor.

*Scarabanema leuckarti* (Hammerschmidt, 1838).

*Synonym*.—*Oxyuris brachyura* Hammerschmidt, 1847.

*Female*.—Body in general cylindrical, neck region distinctly conoid, total length 6.23 to 8.43 mm., maximum diameter 0.423 mm. The nearly cylindrical anterior portion of the esophagus enlarges slightly posteriad, then is abruptly reduced in diameter to form the isthmus. Cardiac bulb oblate. Intestine with club-shaped ventriculus and a lesser posterior dilation. Vulva near middle of body, not salient. Reproductive system double. On either side of the vulva are several (Hammerschmidt figures three) yellowish, spindle-shaped bodies, probably vaginal glands. Eggs ellipsoidal,  $84\mu$  to  $100\mu$  long by  $70\mu$  in breadth.

*Male*.—Unknown.

*Host*.—Believed to have been the larvae of *Rhizotrogus aprilianus* and *R. solstitialis*. The accepted names for these insects are now *Amphimallon assimilis* Hbst. and *A. solstitialis* L., respectively.

*Location*.—Posterior end of intestine (Blinddarm).

*Locality*.—Germany.

Hammerschmidt first described this species as *Oxyuris leuckarti* in 1838 (3). A second paper by this author published in 1847 (4) contains the description of *O. brachyura*. While Hammerschmidt does not specifically state that the two names apply to the same species, a careful examination of his descriptions and figures leaves little room for doubt that *O. brachyura* is *O. leuckarti* renamed and redescribed. One would judge that the yellowish bodies situated near the vulva are fairly conspicuous structures not easily overlooked, and as similar bodies could not be found in *Scarabanema cylindricum*, it is concluded that *S. leuckarti* is specifically distinct.

*Scarabanema brevicaudatum* (Leidy, 1851).

*Synonym*.—*Aorurus (Thelastoma) brevicaudatus* (Leidy, 1851) Walton, 1927 (9).

Body in general cylindrical, rapidly narrowing anteriorly from the cardiac bulb, producing a conical neck region. Body narrows abruptly in anal region, producing a very short spiculate tail. Total length 4.23 mm. (2 lines). Vulva situated slightly posterior to middle of body. Intestine with broad ventriculus and lesser posterior dilation. Anterior portion of esophagus  $0.384$  mm. long by  $38\mu$  in diameter. Cardiac bulb  $0.102$  mm. long by  $89\mu$  in diameter. Ova ellipsoidal,  $89\mu$  long by  $25\mu$  broad.

*Host*.—Larva of *Scarabaeus relictus*. The accepted name for this insect is now *Ligyroides relictus* (Say).

*Location*.—Intestine.

*Locality*.—Near Philadelphia, Pa., U. S. A.



The above diagnosis, taken from Leidy's original description (6) indicates that this parasite belongs to the genus *Scarabanema*, but it is scant information upon which to identify the species. In one particular, however, this species appears to differ from either of the other two species of the genus, namely, in the shape of the ova. While in length (one two-hundred-and-eighty-fifth of an inch or about  $89\mu$ ) they compare favorably with the ova of *S. leuckarti*, they are but  $25.4\mu$  (one-thousandth of an inch) broad, indicating a much narrower and more elongate shape. It seems wiser, therefore, for the present to regard *S. brevicaudatum* as a distinct species.

Hammerschmidt was unable to find males of *Scarabanema leuckarti*, although one would judge from his paper that he made some effort to do so. He expressed the opinion that in some cases the males of insect oxyurids are seasonal in their occurrence. Apparently Leidy was also unsuccessful in finding the males of *S. brevicaudatum*. The writer has, over a period of five years, examined many scarabaeid larvae which were infected with the females of *S. cylindricum* in numbers of from one to eight. Although a careful search was always made, no male was found until one male specimen was secured from a beetle larva collected at Woods Hole, Mass., March 17, 1930.

#### GENUS THELASTOMA LEIDY 1849 (5)

***Thelastoma (Thelastoma) macramphidum*, n. sp. (Figs. 6, 7, and 8.)**

*Female:*

0.6	9.7	22.	'50'	72.	
1.2	4.0	6.0	7.8	3.4	3.5 mm.

Body moderately robust, total length 2.3 to 3.5 mm. Caudal spike 1.1 mm. long from anus. A constriction sets off a distinct head region. (Fig. 6, A.)

The first annule back of the head region is  $13\mu$  to  $16\mu$  wide and the succeeding annules are  $7\mu$  to  $8\mu$ , increasing to  $12\mu$  or  $13\mu$  near the base of the esophagus. Alae are absent. The mouth opening is subtriangular and is bordered by three distinctly elevated lips. (Fig. 7, B and C.) Surrounding the mouth on what may be designated as the outer anterior margin of the head, is a circle of eight elevations which, when viewed from in front (fig. 7, B), appear distinctly oblong, the longest diameter of  $6\mu$  to  $8\mu$  being perpendicular to the corresponding radial axis of the animal. While they are more or less papillalike, they were not observed to possess nerve endings. The two subdorsal and the two subventral elevations are slightly larger than the sublateral.

The amphids are large and conspicuous, and when seen from in front (fig. 7, B) appear as elliptical markings,  $5\mu$  by  $3\mu$ , with the longest diameter dorsoventral. This is probably the outline of the vestibule directly below the cuticle, while the actual opening is considerably smaller and takes the form of a broad slit. When viewed dorsoventrally the amphidial vestibule (fig. 7, C) is seen to possess a very heavy wall. From the base of the vestibule the connection was traced posteriad for only a short distance.

The pharynx (fig. 7, C) is about  $14\mu$  to  $16\mu$  long by  $11\mu$  to  $13\mu$  in diameter. Each of the three sectors of the esophagus is prolonged into a rounded, somewhat inward-pointing projection extending into the base of the pharynx, and each bears on its inner anterior margin a smaller, somewhat rounded, secondary projection (fig. 7, C, t), which might be regarded as a tooth although it does not appear to be very sharp or heavily cuticularized; otherwise the pharynx is plain and unarmed. The anterior portion of the esophagus (fig. 6, A) is of very nearly uniform diameter throughout increasing but slightly and gradually posteriad. The isthmus is separated from it by a distinct constriction and has a diameter very slightly less than that anterior to the constriction.

The intestine is somewhat dilated at its anterior end, forming a ventriculus, but only slightly so at its posterior end. It is not telescoped over the cardiac bulb. The junction of the intestine and rectum is flanked by at least three glands. The anus is not set off by lips. The tail narrows rather abruptly back of the anus and is then extended into a narrow, sharp caudal spike more or less distinctly differentiated from the body proper, closely resembling that of *Thelastoma papilliferum*. (See fig. 9, A.)

The excretory pore is located opposite the cardiac bulb. The ducts of the excretory system are comparatively small and difficult to see in preserved material;

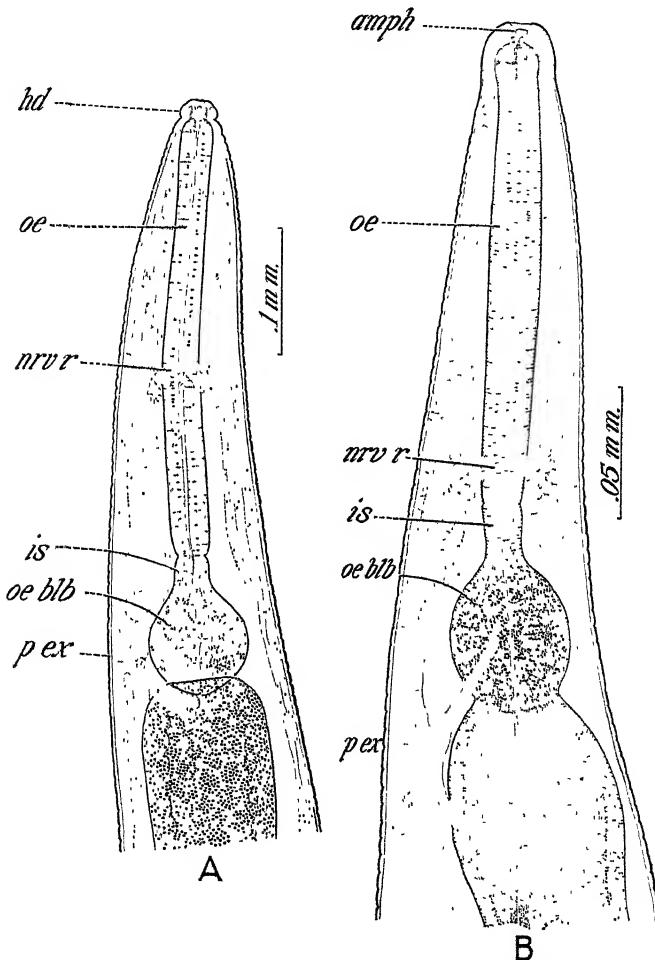


FIGURE 6.—*Thelastoma macramphidum*: A, Anterior end, lateral view, ♀; *hd*, head; *oe*, esophagus; *nr r*, nerve ring; *is*, isthmus; *oe blb*, esophageal bulb; *p ex*, excretory pore; B, anterior end, lateral view, ♂; *amph*, amphid; *oe*, esophagus; *nr r*, nerve ring; *is*, isthmus; *oe blb*, esophageal bulb; *p ex*, excretory pore

there is probably no ampulla. The nerve ring is slightly anterior to the middle of the esophagus.

The protruding vulva can best be understood by reference to Figure 7, A. It is located slightly posterior to the middle of the body (not including the caudal spike). The female reproductive system is double. The vagina is divided near the middle into two portions, a comparatively thin-walled part joining the uteri and a heavy muscular part joining the vulva. The ova are ellipsoidal,  $80\mu$  by  $60\mu$  in diameter, and are deposited before segmentation.

## Male.

0.61	13.	20.	'M	83.	
0.43	6.0	7.00	8.0	3.0	1.45 mm.

Body moderately robust, total length 1 to 1.4 mm.; diameter at middle of body,  $58\mu$  to  $90\mu$ ; annulation coarse and fairly conspicuous, averaging  $5\mu$  to  $7\mu$  in width. Narrow alae are present,  $4\mu$  wide at middle of body. A head region is set off by a constriction somewhat wider and shallower than that in the female. The mouth opening is triangular and without distinct lips. The lobelike projections occurring on the head of the female could not be definitely seen on the male. It is not improbable that these projections do occur on the male; in fact, what may have been four, or even eight, very small elevations (fig. 8, B) were observed. The amphids are large and conspicuous, the elliptical external manifestations measuring about  $4.5\mu$  by  $2\mu$ . The vestibule wall is not so heavily cuticularized as in the female. The anterior portion of the esophagus (fig. 6, B) is expanded slightly in the middle, where it has a diameter about three-eighths that of the corresponding body diameter. It gradually tapers both anteriad and posteriad. The

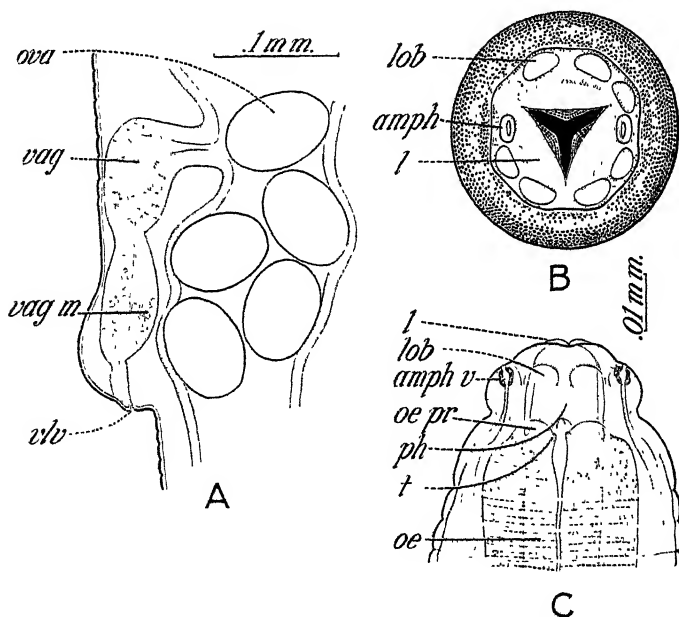


FIGURE 7.—*Thelastoma macramphidum*: A, Vulva and vagina, lateral view; *ova*, *ova*; *vag*, vagina, *vag m*, muscular portion of vagina; *vul*, vulva; B, end view of head, ♀; *lob*, lobe; *amph*, amphid; *l*, lip; C, dorsal view of head, ♀; *lob*, lobe; *amph*, amphid; *oe pr*, esophageal projection, *ph*, pharynx; *t*, tooth, *oe*, esophagus

isthmus is a little longer than in the female, the cardiac bulb a little more elongated, and the excretory pore situated a little farther back. The nerve ring is but slightly anterior to the isthmus, being considerably farther back than in the female. The intestine is dilated anteriorly to form a ventriculus.

The shape of the tail of the male is shown in Figure 8, A and C. The anus is located on a distinct elevation and is surrounded by five (or possibly they should be considered as six) papillae; a pair of preanal papillae, one on the right side and one on the left, situated rather close together; a pair about opposite the anus separated a little farther from one another; and a median, postanal, duplex papilla. The two nerve endings present in this last-mentioned papilla indicate that it is really double, probably representing an anastomosed pair. A pair of papillae also occur on the tail about  $80\mu$  from the anus, i. e., just behind the beginning of the middle third. The length of the tail, however, is variable. Even in different individuals of approximately the same body size it may differ considerably. The preanal papillae are about  $5\mu$  long and  $4\mu$  wide, those opposite the anus and

those on the tail about the same size, possibly a little smaller. The latter are not always in the same transverse plane. The spicule (fig. 8, A) varies in length from  $40\mu$  to  $55\mu$  with a diameter of from  $3.5\mu$  to  $5\mu$  in its thickest region, which is about one-third the distance from its proximal end. It is slightly expanded at its proximal end, and ends distally in a ball-like enlargement a micron or more in diameter. The testis is large and reflexed.

*Host*.—Larva of *Osmoderma* (*O. scabra* Beauv.?).

*Location*.—Posterior end of intestine.

*Locality*.—Near Ann Arbor, Mich., U. S. A.

*Type specimens*.—United States National Museum Helminthological Collections No. 8110.

*Paratypes*.—No. 8111.

*Technic*.—Material killed in hot Flemming's strong, preserved in 4 per cent formalin, and cleared in glycerin. All observations and measurements made on specimens so treated.

***Thelastoma (Thelastoma) papilliferum*, n. sp.**  
(Figs. 9, 10, and 11.)

*Female*.

0.4 8.0 17. '46' 72.  
0.8 4.0 5.6 6.7 4.0 3.9 mm.

Body moderately robust, total length 3.25 to 4.25 mm., diameter at middle of body  $0.18\mu$  to  $0.28\mu$ , caudal spike 0.45 to 0.9 mm. long from the anus. First annule back of the head  $18\mu$  wide, succeeding annules  $7\mu$ , increasing gradually to  $12\mu$  in middle of body. Alae are not present. Seen from in front the amphids appear oblong in shape, being about  $3\mu$  by  $2\mu$ . These measurements represent the outline of the vestibule seen under the cuticle, the opening being a broad slit about  $1.5\mu$  long. The head is rounded and set off by a wide constriction. The triangular mouth is bordered by three slightly arched lips (fig. 9, C), each lip being faintly divided into two parts. On the anterior outer margin of the head is a circle of eight elevations, distinctly innervated and in every respect papilloid in character. Each is about  $4\mu$  in height and slightly oblong in optical cross section at its base, with a greater diameter of  $4\mu$ . There extend into the base of the pharynx three comparatively large,

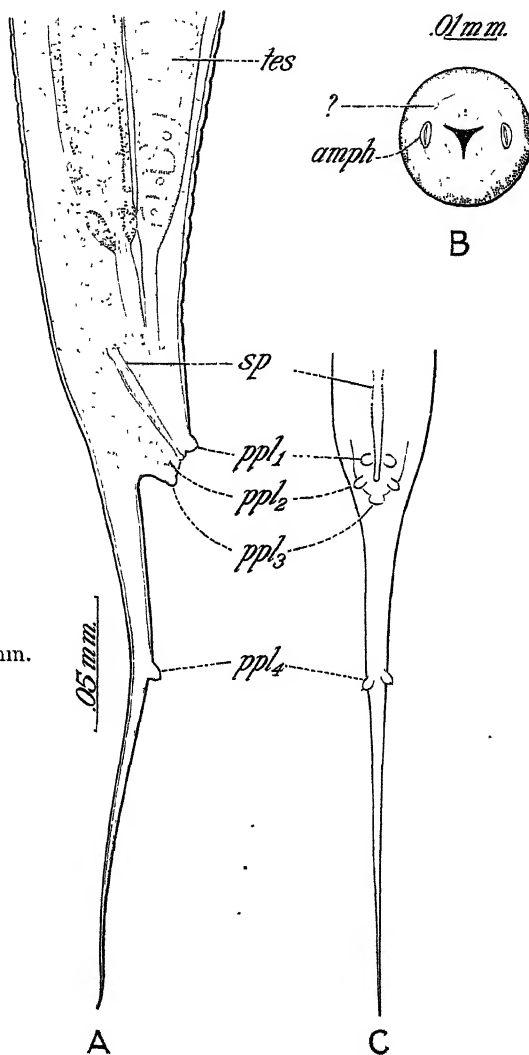


FIGURE 8.—*Thelastoma macramphidum*: A, Posterior end, lateral view,  $\delta$ ; tes, testis; sp, spicule; ppl<sub>1</sub>, preanal papillae, ppl<sub>2</sub>, papillae opposite anus; ppl<sub>3</sub>, median, postanal, duplex papilla; ppl<sub>4</sub>, papillae on caudal spike; B, end view of head,  $\delta$ ; (?), small elevations; amph, amphids; C, posterior end, ventral view,  $\delta$ ; sp, spicule; ppl<sub>1</sub>, preanal papillae; ppl<sub>2</sub>, papillae opposite anus, ppl<sub>3</sub>, median, duplex, postanal papilla, ppl<sub>4</sub>, papillae on caudal spike

rather blunt, more or less toothlike projections. (Fig. 9, C.) The anterior portion of the esophagus is nearly cylindrical, and the isthmus is set off by a constriction and is but slightly reduced in diameter. The intestine is slightly dilated anteriorly.

The excretory pore is opposite the cardiac bulb. The nerve ring is located posteriad about three-fifths the distance from the pharynx to the isthmus.

The vulva (fig. 10) is located slightly posterior to the middle of the body (not including the terminal spike) and is set off by small lips, but does not protrude. The vagina is short and about 0.1 mm. in length, extends anteriorad, and is divided

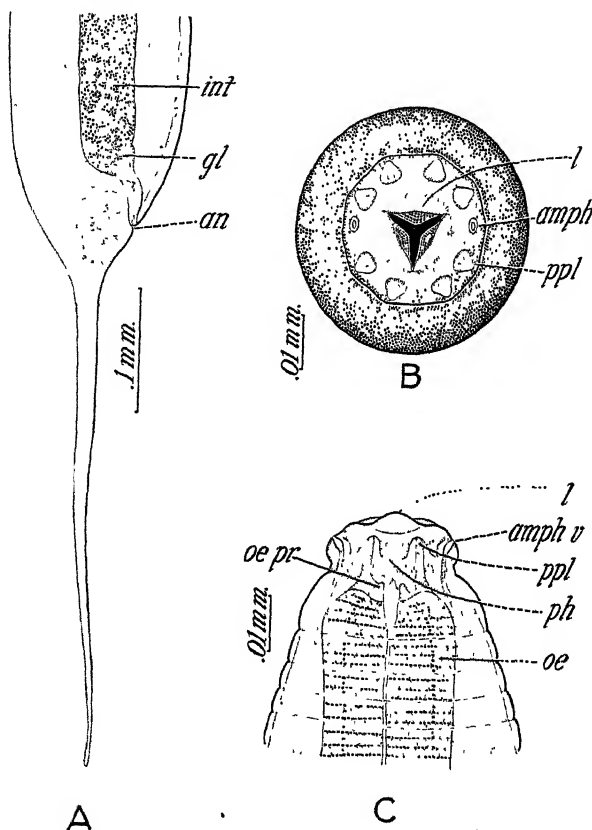


FIGURE 9.—*Thelastoma papilliferum*: A, Posterior end, lateral view, ♀; int, intestine; gl, gland; an, anus; B, end view of head, ♀; l, lip; amph, amphids; ppl, papillae. C, dorsal view of head, ♀; oe pr, esophageal projection; l, lip; amph v, amphidial vestibule; ppl, papillae; ph, pharynx; oe, esophagus

into two regions. That next to the uteri constitutes about two-thirds of the total length, has a diameter of  $35\mu$ , is thick-walled and not predominantly muscular. The remaining third has a diameter about half as great, a thick muscular wall, a narrow and more heavily cuticularized lumen, and is surrounded by a hemispherical mass of muscular tissue. On the right side of the vagina near the vulva and embedded in the surrounding tissue are five or six unicellular structures,  $10\mu$  to  $15\mu$  in diameter, which resemble glands. The reproductive system is double. The ova are ellipsoidal ( $80\mu$  by  $60\mu$ ) and are deposited before segmentation.

## Male:

0.36	13	19	'M	80	1.12mm.
0.36	5.5	6.5	7.5	3.5	

Body moderately robust, total length 1 to 1.2 mm.; diameter in middle of body  $60\mu$  to  $74\mu$ , caudal spike 0.19 to 0.22 mm. long from anus. The head (fig. 4, B) is very faintly set off by a broad, almost imperceptible constriction, and adjacent to it there appears to be a wide annule, but the striae near the head are not distinct. The annules are  $6\mu$  wide near the middle of the body. Alae are present  $4\mu$  wide at the middle of the body. The amphids are relatively small, with the external manifestation elliptical. The eight papillalike structures characteristic of the female were not seen on the head of the male. The anterior portion of the esophagus (fig. 11, A) is nearly cylindrical throughout, and the isthmus is set off by a fairly distinct constriction. The cardiac bulb is broadly pyriform and the valvular apparatus relatively large and distinct. The intestine is dilated anteriorly to form a ventriculus.

The excretory pore is slightly posterior to the cardiac bulb, and the nerve ring is at the anterior end of the isthmus.

The anus is located on a distinct elevation, and there are five circumanal papillae: One pair slightly preanal and rather close together (fig. 11, B); one pair opposite the anus and somewhat more widely separated; and a single median, duplex, postanal papilla. The spicule is very faintly expanded at the proximal end and slightly expanded at the tip. The tail tapers gradually throughout its length and ends in a sharp point. Somewhat more than one-third the distance from the anus to the tip it bears a pair of papillae slightly larger than the preanal papillae, and at this point usually bends abruptly dorsad. The testis is large and reflexed.

*Host*.—Larva of *Osmoderma* (*O. scabra* Beauv.?).

*Location*.—Posterior end of intestine.

*Locality*.—Near Ann Arbor, Mich., U. S. A.

*Type specimens*.—United States National Museum Helminthological Collection No. 8112.

*Paratypes*.—No. 8113.

This species resembles *Thelastoma macramphidum*, but differs from it in the following respects: In the female the cephalic elevations are more papilloid in character and distinctly innervated, the amphids smaller with the amphidial vestibule less heavily cuticularized; and the vulva is situated a little farther forward and is not salient. In the male the amphids are considerably smaller, and the anal elevation somewhat more pronounced.

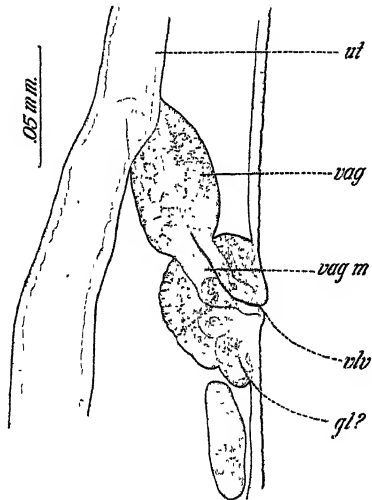


FIGURE 10.—*Thelastoma papilliferum*, vulva and vagina, lateral view; *ut*, uterus; *vag*, vagina; *vag m*, muscular portion of vagina, *vle*, vulva, *gl?* gland?

## GENUS AORURUS LEIDY 1849

*Aorurus subcloatus*, n. sp. (Figs. 12, 13, and 14.)

## Female:

0.16	1.7	9.0	'57'	58	3.05 mm.
0.85	2.3	5.3	4.0	2.6	

Body robust, total length 2.3 to 3.5 mm., caudal spike 1 to 1.2 mm. long from anus. Annulation coarse, very conspicuous; annules  $10\mu$  wide back of head region, increasing to  $20\mu$  at middle of body. Narrow alae are present. The head (fig. 12, A) is more or less truncate and very short. The circular to sub-

triangular mouth is not bordered by lips. The amphids are located on the outer anterior margin of the head. The conspicuous external manifestations (fig. 13, B) are elliptical,  $3.5$  by  $2\mu$ . This is likewise probably the outline of the vestibule below the cuticle, the openings being obscure. When seen in dorsoventral aspect, the vestibule (fig. 13, C) is  $2\mu$  wide by  $3$  to  $3.5\mu$  long, with a wall not heavily cuticularized. Surrounding the mouth, in a circle with the amphids, are eight slightly elevated, somewhat incurved lobes. When seen from in front these lobelike elevations are about  $6\mu$  broad and somewhat rectangular in outline. They were not observed to be innervated. The pharynx (fig. 13, C) is

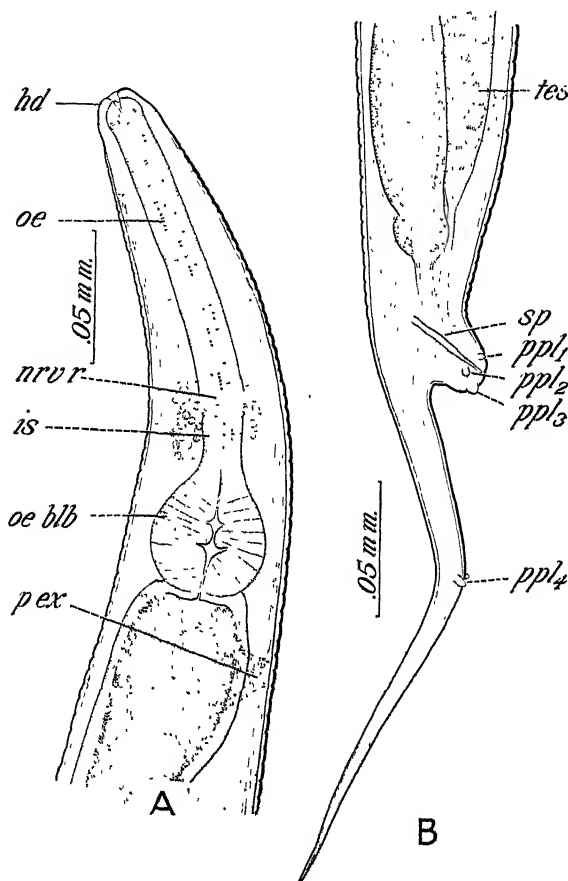


FIGURE 11.—*Thelastoma papilliferum*: A, Anterior end, lateral view, ♂; *hd*, head; *oe*, esophagus; *nrv r*, nerve ring; *is*, isthmus; *oe blb*, esophageal bulb, *p ex*, excretory pore; B, posterior end, lateral view, ♂, *tes*, testis; *sp*, spicule; *ppl*<sub>1</sub>, papillae slightly preanal; *ppl*<sub>2</sub>, papillae opposite anus; *ppl*<sub>3</sub>, median, duplex, postanal papilla; *ppl*<sub>4</sub>, papillae on caudal spike

about  $5\mu$  deep by  $9\mu$  wide, and unarmed. While the inner anterior margins of the three segments of the esophagus are somewhat angular, they are not toothlike.

The esophagus is short ( $0.22$  mm.), and the anterior portion is enlarged into a distinct, pyriform median bulb (fig. 12, A), the diameter of which ( $70\mu$ ) is nearly as great as that of the cardiac bulb. The isthmus is short, distinct, and slightly expanded in the middle. The cardiac bulb is broadly pyriform and rather dense with granular material. It opens posteriad through a slightly projecting cardia into the large ventriculus. The latter has a maximum diameter four-fifths as great as the corresponding body diameter and tapers gradually posteriad. The posterior end of the intestine is not dilated.

The rectum (fig. 14) is conoid, has a length a little greater than the anal body diameter, and possesses a narrow cuticularized lumen. While rectal glands were not seen and consequently not figured, they are very likely present. The tail averages 1 mm. in length, or over 30 per cent of the total body length. It is  $7\mu$  to  $8\mu$  in diameter where it joins the body and tapers gradually to a fine point.

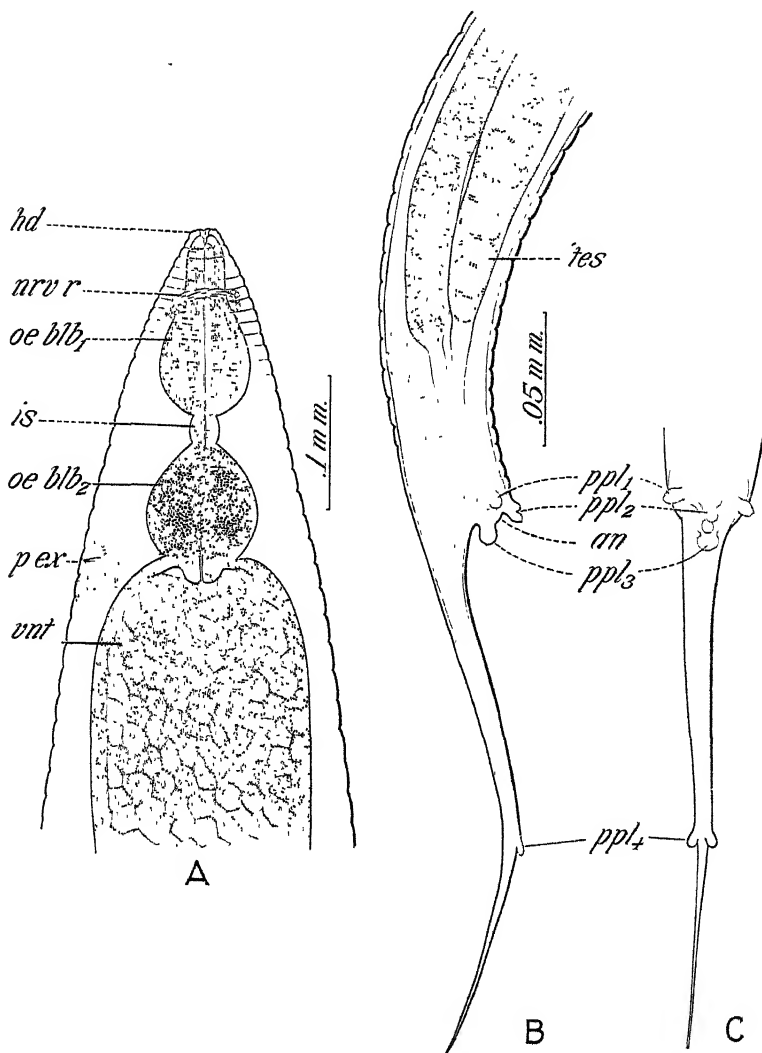


FIGURE 12.—*Iorurus subeloatus*: A, Anterior end, lateral view, ♀; *hd*, head, *nrv r*, nerve ring; *oe blb1*, median bulb; *oe blb2*, cardiac bulb, *is*, isthmus, *per*, excretory pore, *vnt*, ventriculus; B, posterior end, lateral view, ♂; *tes*, testis; *ppl1*, papillae on sides of anal elevation; *ppl2*, submedian, preanal papillae, *ppl3*, median, duplex, postanal papilla; *ppl4*, papillae on caudal spike; *an*, anus; C, posterior end, ventral view, ♂ (legends as in B)

The nerve ring (fig. 12, A) encircles the neck of the median bulb. The excretory pore is opposite the cardiac bulb.

Measured on the outer contour of the body, the vulva (fig. 14) is located about  $30\mu$  anterior to the anus and is slightly salient. The vagina extends anteriorly about one-third the distance from the anus to the head, where it branches to form the two uteri. The posterior end for a distance of  $90\mu$  is differentiated into



a muscular-walled region with a narrow lumen, while the remainder is a thin-walled tube with a wide lumen. The organization of the remainder of the female reproductive system is very similar to that in all the other species dealt with in this paper. The shifting of the vulva posteriad does not materially affect the position of the uteri and ovaries.

The ellipsoidal ova,  $90\mu$  long by  $60\mu$  broad, are not noticeably flattened on one side, and are deposited before segmentation begins.

Male:

0.74	13	19.	'M	80.	1.08 mm.
0.76	5.0	5.3	5.3	3.5	

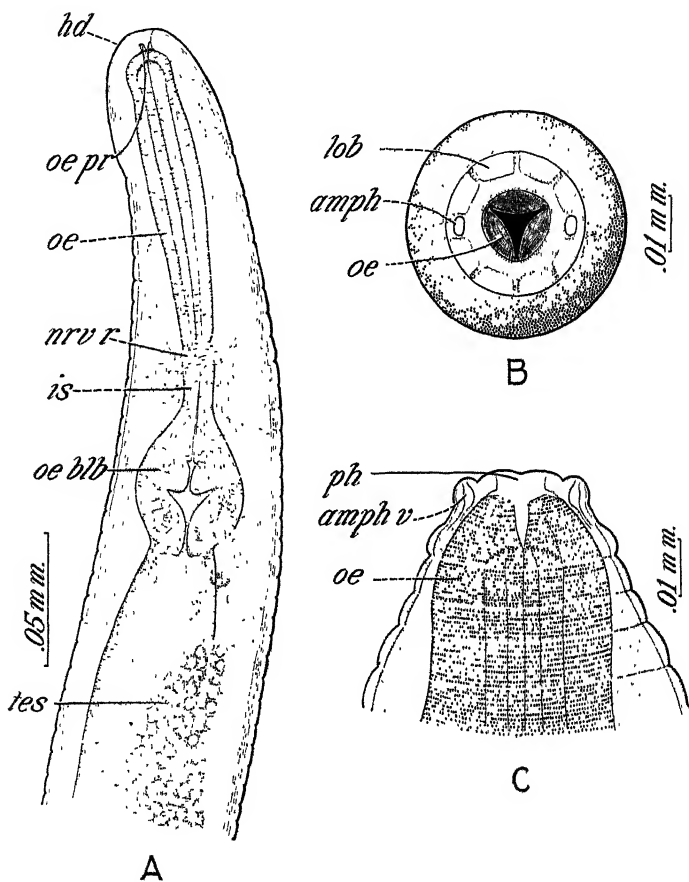


FIGURE 13.—*Aorurus subeloatus*: A, Anterior end, lateral view, ♂, *hd*, head; *oe pr*, esophageal projections; *oe*, esophagus; *nrv r*, nerve ring; *is*, isthmus; *oe blb*, esophageal bulb; *tes*, testis, B, end view of head, ♀: *lob*, lobe, *amph*, amphid; *oe*, esophagus; C, dorsal view of head, ♀, *ph*, pharynx; *amph v*, amphidial vestibule, *oe*, esophagus

Body moderately slender, total length 0.84 to 1.1 mm., caudal spike 0.15 to 0.18 mm. long. Annulation coarse and conspicuous, annules  $5\mu$  wide back of headcap, increasing to  $6\mu$  or  $8\mu$  in middle of body. Narrow alae present,  $6\mu$  wide in middle of body. The head (fig. 13, A) is rounded, and lacks the lobelike elevations of the female. The amphids are similar in shape to those of the femlae, but smaller and less conspicuous. The mouth is subtriangular and not bordered by lips. Posteriad from the mouth for a distance of  $50\mu$  the anterior end lacks transverse striae, forming a smooth, slightly expanded headcap. (Fig. 13, A.) The pharynx is  $8\mu$  deep with an average diameter of  $6\mu$ . There protrudes into it from each of the three sectors of the anterior end of the esophagus a small, somewhat toothlike projection.

The anterior portion of the esophagus expands gradually posteriad to a point opposite the posterior margin of the headcap, where its diameter is  $22\mu$  to  $25\mu$ , then gradually diminishes in size until, adjacent to the isthmus, its diameter ( $14\mu$  to  $18\mu$ ) is less than at the anterior end. While it is thus noticeably expanded, it does not possess any suggestion of a median bulb. The isthmus is set off anteriorly by a distinct constriction, is about  $28\mu$  long, and has a diameter of from  $12\mu$  to  $14\mu$ . The cardiac bulb is slightly longer than broad and possesses a very large and conspicuous valvular apparatus. The intestine may be somewhat dilated anteriorly.

The excretory pore is slightly posterior to the cardiac bulb; the nerve ring is at the anterior end of the isthmus.

The anus (fig. 12, B) is located on an elevation, and is surrounded by five circumanal papillae. There is a median pair of preanal papillae (fig. 12, B and C), more or less joined at their base and in many specimens so arranged as to appear one behind the other. There is also a pair (one right and one left) of more widely separated, somewhat preanal papillae occurring on the sides of the anal elevation, and a median, duplex, postanal papilla with two nerve endings. Another pair occurs on the tail about three-fifths the distance from the anus to the tip. All the papillae are somewhat larger than those of *Thelastoma macramphidum*. The four preanal papillae are  $8\mu$  long and  $5.5\mu$  to  $6\mu$  wide, while the postanal papilla is about  $8\mu$  long and  $8\mu$  wide when seen in dorsoventral aspect. *Host*.—Larva of *Osmoderma* (*O. scabra* Beauv.?).

*Location*.—Posterior end of intestine.

*Locality*.—Near Ann Arbor, Mich., U. S. A.

*Type specimens*.—United States National Museum Helminthological Collections No. 8114.

*Paratypes*.—No. 8115.

*Technic*.—Material killed in warm Flemming's strong, preserved in 4 per cent formalin, and cleared in glycerin. All observations and measurements were made on specimens so treated.

The females of this species resemble those of *Aorurus agile* Leidy, 1849, but are at once distinguished from them by the close proximity of the vulva to the anus.

The papillae on the tail of the male are situated considerably farther back than the corresponding papillae on the tail of *Thelastoma macramphidum* or *T. papilliferum*. A very distinct cuticularization was noted near the anus, which may have been a rudimentary spicule or the wall of the cloaca. In several cases a very small, indistinct projection was noted protruding from what appeared to be the lip posterior to the anus or possibly through the anus itself. Whether or not this was connected with the cuticularization within could not be determined from the specimens available. In dorsoventral view this projection was obscured by the preanal papillae.

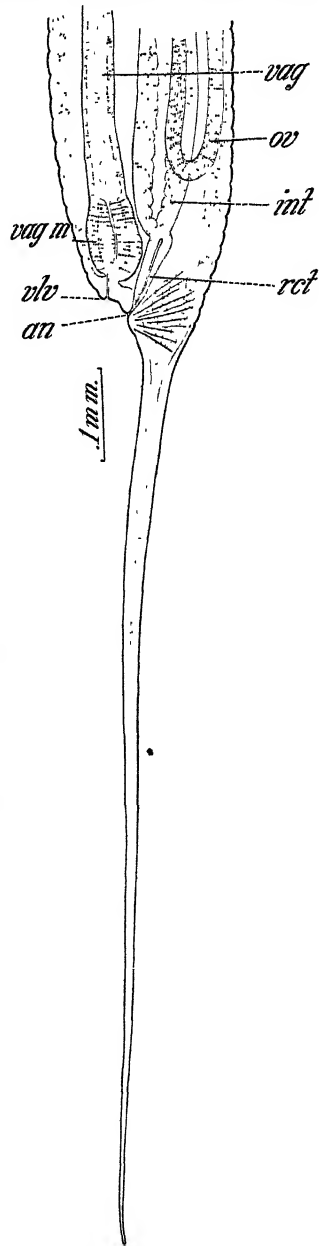


FIGURE 14.—*Aorurus subeloatus*, posterior end, lateral view, ♀; *vag m*, muscular portion of vagina; *vul*, vulva; *an*, anus; *vag*, vagina; *ov*, ovary; *int*, intestine; *rect*, rectum

## SUMMARY

Four new oxyurid parasites of scarabaeid larvae are described. One species from an unidentified scarabaeid larva belonging either to the Rutelinae or the Melolanthinae is placed in a new genus, *Scarabanema*. Two new oxyurid parasites from the larva of a species of *Osomoderma* (*O. scabra* Beauv.?) are placed in the genus *Thelastoma* Leidy, and one new species from the same host is placed in the genus *Aorurus* Leidy. Keys to the genera and species are given.

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# GROWTH HABIT AND YIELD IN WHEAT AS INFLUENCED BY TIME OF SEEDING<sup>1</sup>

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## INTRODUCTION

Almost the entire wheat crop of eastern Oregon was destroyed by freezing in December, 1924. A preliminary date-of-seeding experiment conducted at the Sherman County branch station, Moro, Oreg., during 1923-24 had shown that winter wheat could be sown as late as February 15 and still head and produce yields equal to or better than those of spring varieties. Farmers were advised of this fact, and many of them took advantage of favorable weather in late January and early February, 1925, and reseeded their land to winter varieties, with good results. The experiments were enlarged and continued to obtain more complete data as to the latest date at which winter varieties could be sown and still yield as well as spring varieties, or even produce heads and seed.

## REVIEW OF LITERATURE

Aamodt (1)<sup>4</sup> states that Kanred winter wheat sown in the spring at St. Paul, Minn., produced an occasional head late in the season but did not set seed. Gaines (8) found that winter barley tillered profusely but did not head when spring sown at Pullman, Wash. Schafer et al. (14) reported that Hybrid 128 winter wheat sown after March 11 would not head until the second season. Call and Salmon (4) reported that wheat sown in Kansas in February produced a fair yield in some cases. Seiger,<sup>5</sup> a farmer at Malin in Klamath County, Oreg., obtained a yield of 36 bushels per acre from Turkey wheat sown March 15, whereas the same variety sown April 10 did not head. This was at an altitude of about 4,500 feet where the growing season is short. Breithaupt (3) states that winter rye sown in south central Oregon as late as April 1 may produce fair yields, although a delay of one or two weeks in the time of seeding may result in failure.

Percival (13), in England, found that all winter-wheat varieties sown before the end of March produced ears the same season, but that after this period there is a critical date for each variety. If sown after this critical date, the plants of any winter variety continue prostrate vegetative growth during the summer and head during the second year, if not killed by drought or other unfavorable conditions. Percival found that a given variety sown on the same date headed on nearly the same date each year. Different varieties sown on each of several dates maintained relative heading dates.

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<sup>3</sup> Assistant in the cooperative experiments at the Sherman County branch station, Moro, Oreg.

<sup>4</sup> Reference is made by number (italic) to Literature Cited, p. 499.

<sup>5</sup> SEIGER. [WHEAT LETTER FROM MALIN, OREG.] Letter on file in Farm Crops Dept., Oregon Agr. Col. 1924.

Adams (2) seeded Kharkof winter wheat and winter rye in pots in the greenhouse at Ottawa, Canada, at weekly intervals from March 5 to April 23, 1923. The plants were transplanted outside May 15. All plants sown in March headed out and completed their life cycle the same season. Plants sown in April in most cases did not produce heads until the next season, when they headed normally. With rye the difference in growth from the different dates of seeding was not marked. A few heads were produced from each date of seeding except one in 1923, but plants from each date of seeding lived over and produced heads in 1924.

Kiesselbach (12) sowed Nebraska 60, a strain of Turkey wheat, at Lincoln, Nebr., on different dates from September to late March in the years 1919 to 1923, inclusive. No heads were produced from the crop sown March 4 to 26 in four of the years, and only a half bushel of grain per acre in the fifth year. The crop sown March 2 to 11 in the same years produced no grain in two of the years and an average of 9.1 bushels for the other three years. The crop sown from January 25 to February 18 produced grain each year, averaging 11.8 bushels. Sowings made September 20 to 25 averaged 34.2 bushels per acre.

Garner and Allard (9) found that by varying the daily exposure to light through the use of electric lights in the greenhouse many plants of different species could be made to flower and produce seed at any period of the year. They suggested that length of day and not temperature determines the time when flowering will occur. They divided plants into two groups, "short day" plants, which flower and fruit as the days grow shorter, and "long day" plants, which flower and fruit as days grow longer. They placed the cereals in the "long day" group.

Following up the work of Garner and Allard, Wanser (16, p. 314) suggested that one ratio of daylight to darkness may cause a variety of wheat to joint and another ratio may cause it to head. He suggested that the photoperiods causing jointing in spring-wheat varieties are of greater magnitude than for winter varieties and possibly without a maximum limit. To quote: "Photoperiodism, therefore, is the key to the distinction between winter and spring wheats." He also suggested that the northern limit of the winter-wheat belt is controlled largely by the relation between the date when active growth begins and the date on which the longest day within the limit of the critical photoperiod for jointing occurs. Cooper (5) states that winter wheat will not head when sown in the spring, because it requires a long vegetative period, and by the time this is over the duration of daily sunlight is too short to bring the plants into heading. Artificial light greatly reduced the growing period of wheat sown early in the greenhouse. There was little effect, however, on wheat sown as late as the middle of February, because the artificial light had less effect as the days became longer in the spring.

It was once supposed that winter varieties of the cereal crops must pass through a period of low temperature after germination before they could head. Recent experiments have shown that a freezing temperature is not necessary. Fruwirth (?) states that in true winter varieties no stalks will ripen, or at most only a few, if the seed is sown in March or later. If the plants from later sown seed are exposed for a sufficient length of time to temperatures lower than

the ones to which the older plants are subjected they will produce heads and will ripen. They need not be exposed to frost, however. Call and Salmon (4) state that after winter wheat has germinated it must pass through a cool period but not a freezing one before it will head.

Hutcheson and Quantz (11) grew winter varieties of wheat, oats, barley, and rye in greenhouses kept at 75°, 65°, 62°, and 58° F. Wheat showed striking differences in growth as a result of variation in temperature, producing a normal growth in the cool house but only a mass of leaves in the two warmest houses.

Gassner (10) germinated seeds of winter and spring varieties of rye, wheat, and barley at 1°–2°, 5°–6°, 12°, and 24° C. on each of several dates and then set the plants outdoors under uniform conditions. All plants of spring varieties, from seeds germinating at the same time, even under different temperatures, headed at the same time. Plants of winter varieties from seeds germinated at the lower temperatures headed sooner. Thus, plants from seeds germinated during the middle of March at 1°–2° C. formed heads 9, 21, and 41 days earlier, respectively, than the plants from seeds germinated at the same time but at 5°–6°, 12°, and 24° C. The later the date of seeding the lower was the temperature required at germination for winter varieties to head. Gassner believed that low temperature had a positive effect even when limited to the germination phase, and that winter wheat, rye, and barley will produce grain when spring sown if subjected to a sufficiently low temperature during germination.

Dickson (6) grew Turkey wheat (Wisconsin Pedigree No. 2) in the greenhouse from October until the following summer in soils in which temperatures varied at 4° intervals from 8° to 36° C. The plants grown in soil held at temperatures of 24° C. and higher produced an excessive vegetative growth with little or no formation of heads. When grown in soils held at temperatures of 20° C. and lower the plants headed normally during May. Chemical analyses showed that the plants grown at low temperatures were high in total carbohydrates and low in total nitrogen, whereas the reverse was true at higher temperatures. Strains of Turkey and White Winter wheat were grown to the first-leaf stage in the greenhouse at 5° C. and then transplanted to the field in April. Seed from the same strains was sown in the field at the same time. Of the Turkey plants grown from seed germinated in the greenhouse at 5° C., 62 per cent headed and formed grain. The Turkey plants grown from seed sown in the field made an excessive vegetative growth, but formed no heads. Among 12 varieties seed of which was germinated at 5° C. and transplanted to the field in April, all gradations in heading occurred. No heads were formed in some varieties, whereas in others 96 per cent of the plants formed heads. Dickson also found chemical differences in plants at the time of emergence when they were germinated at different temperatures, there being a decline in carbohydrates and an increase in total nitrogen when the seeds were germinated at higher temperatures. Wheat plants showed the effect of the germinating temperatures in the growth subsequent to the seedling stage even when this growth was made under uniform conditions.

Walster (15) planted Oderbrucker barley in pots on March 1. Part of the pots were kept at 15° and part at 20° C. He varied the nitrogen, phosphorus, and potassium in the nutrient solution added

to the pots. All plants grown at the low temperature headed normally, but only those receiving a low supply of nitrogen headed in the warmer houses. He believed a low carbohydrate-nitrogen ratio in the plant tissues turned the plants toward vegetative growth.

#### VARIETIES AND SOWING DATES

The experiments reported herein were conducted at Moro, Oreg., in the years 1925, 1926, and 1927. Thirteen varieties of wheat were sown in 1925 on several dates, ranging from February 2 to April 23; 16 varieties the following year, ranging from October 14, 1925, to June 4, 1926; and 8 in the third year, ranging from October 20, 1926, to May 31, 1927. The intervals varied from one to two weeks from February 1 to May 1, the period during which critical dates for the winter varieties occurred, but were longer during the early part of the winter. Varieties were chosen which represented a wide range of type. They included 11 typical winter varieties, 3 typical spring varieties, and 6 which may be classed as intermediate. A supplementary test including Marquis, Hard Federation, Quality, and Sunset was sown on July 6, 1927. Yields were not obtained on this seeding, as the varieties were injured by stock. Each variety was sown in two 3-row plots 1 rod long on each date. A brief description of each variety grown is given in Table 1.

TABLE 1.—Description of the wheat varieties studied

Variety	C. I. No. <sup>a</sup>	Head type <sup>b</sup>	Species	Grain color	Growth habit
Kharkof.....	8249	BSW	<i>Triticum vulgare</i> ..	Red.....	Winter.
Oro.....	8220	BSW	do.....	do.....	Do.
Regal.....	7364	BSW	do.....	do.....	Do.
Kanred.....	5146	BSW	do.....	do.....	Do.
Blackhull.....	6251	BSW	do.....	do.....	Do.
Turkey.....	7396	BSW	do.....	do.....	Do.
Rudit.....	6703	ASW	do.....	do.....	Do.
Triplet.....	5408	APW	do.....	do.....	Do.
White Odessa.....	4655	ASR	do.....	White.....	Do.
Fortyfold.....	4156	ASR	do.....	do.....	Do.
Hybrid 128.....	4512	ASW	<i>Triticum compac-</i> <i>tum</i> .....	do.....	Do.
Hybrid 63.....	—	ASW	do.....	do.....	Intermediate.
Hybrid 143.....	4513	ASW	do.....	do.....	Do.
Federation.....	4734	ASR	<i>Triticum vulgare</i> ..	do.....	Do.
Pacific Bluestem.....	4067	ASW	do.....	do.....	Do.
Jenkin.....	—	ASR	<i>Triticum compac-</i> <i>tum</i> .....	do.....	Spring.
Galgals 39.....	—	APR	<i>Triticum vulgare</i> ..	do.....	Do.
Marquis.....	4158	ASW	do.....	Red.....	Do.
Baart.....	1897	BSW	do.....	White.....	Do.
Hard Federation.....	4733	ASR	do.....	do.....	Do.
Quality.....	6607	ASW	do.....	do.....	Do.
Sunset.....	3728	ASW	do.....	do.....	Do.

<sup>a</sup> Accession number of the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture

<sup>b</sup> B=bearded, A=awnless, S=smooth chaff, P=pubescent chaff, W=white chaff, R=red chaff.

## CLIMATIC CONDITIONS

Winterkilling of ordinarily hardy winter wheat is slight at Moro, and the temperatures usually are such that wheat will germinate if sown at any time during the winter. Good yields often are obtained from winter seeding.

The crop year of 1925 in eastern Oregon was unusual. Almost the entire wheat crop was killed in December, 1924, but temperatures were above normal after the middle of January, permitting seeding in late January and February. No winterkilling, even of tender spring varieties, occurred in 1925-26. Temperatures were above

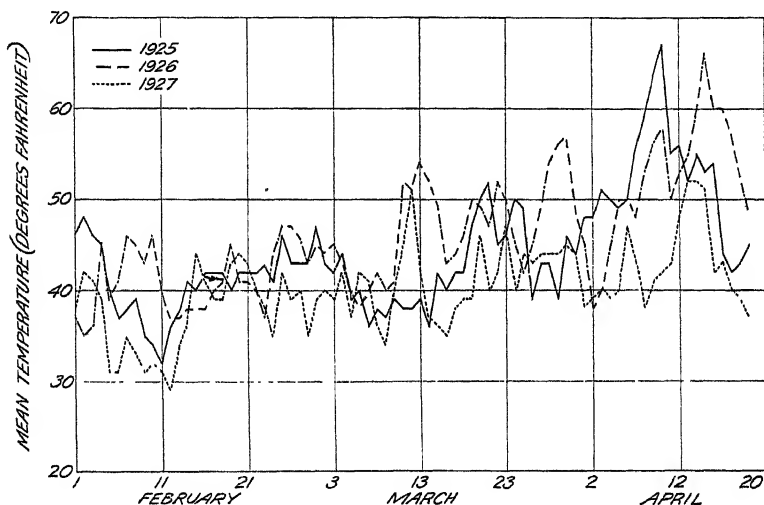


FIGURE 1.—Mean daily temperature, February 1 to April 20, in the years 1925, 1926, and 1927, at Moro, Oreg.

normal all during the winter. All varieties matured unusually early. No killing of winter varieties occurred in 1927, but some spring varieties sown October 20 were injured. These varieties sown on this date headed later than when sown during the late winter. The spring of 1927 was very late and cool, and the precipitation was above normal.

The maximum, minimum, and mean air temperatures, with departures from normal, number of clear days, and precipitation for the three crop years are given by months in Table 2. Figure 1 shows the mean daily temperature from February 1 to April 20 for each year.



TABLE 3.—Dates first and fully headed, plant height, yield, and bushed weight of 13 varieties of wheat seeded in nursery rows at intervals during the season of 1924-25 at Moro, Oreg.

Item and variety	C. I. No.	Date sown (first) and date emerged* (second)							
		Feb. 2 Mar. 19	Feb. 16 Mar. 20	Feb. 26 Mar. 24	Mar. 10 Mar. 31	Mar. 20 Apr. 5	Apr. 2 Apr. 12	Apr. 13 Apr. 27	Apr. 23 May 2
Date first headed:									
Hybrid 128	4512	June 15	June 19	June 23	June 24	June 23	July 3	(b)	(b)
Kharof	8249	June 11	June 16	June 17	June 20	June 20	July 30	(b)	(b)
Kanad	5146	do.	June 15	June 16	June 20	do.	do.	(b)	(b)
Turkey	7366	do.	June 14	June 16	June 20	June 26	do.	July 9	(b)
Pacific Bluestem.	4067	May 31	June 5	June 7	June 12	June 18	June 23	June 27	June 28
Federation	4734	May 26	May 28	May 28	June 2	June 10	June 19	June 22	June 28
Hybrid 143	4513	June 7	June 12	June 16	June 20	June 21	June 26	July 1	July 9
Hybrid 63		June 12	June 15	June 17	do.	June 23	do.	Do	July 7
Jenkin.		June 13	do.	do.	do.	June 21	June 25	do.	July 7
Marquis.	4158		June 6	June 7	June 11	June 15	June 19	do.	June 24
Galgalos 39		June 2	June 6	do.	do.	June 9	June 16	do.	June 27
Baart.	1697			May 27	June 2	June 9	June 16	do.	June 27
Hard Federation.	4733			May 21	May 25	May 28	June 5	June 15	June 20
Date fully headed									
Hybrid 128	4512	June 24	June 28	June 28	July 1	July 2	July 1	July 10	July 16
Kharof	8249	June 25	June 25	June 26	June 27	June 27	July 1	July 10	July 16
Kanad	5146	do.	do.	June 24	June 27	June 27	July 6	July 1	July 16
Turkey	7366	June 22	June 23	June 24	June 27	June 27	July 6	July 1	July 16
Pacific Bluestem.	4067	June 13	June 12	June 17	June 22	June 21	June 28	July 1	July 16
Federation	4734	June 8	June 6	June 9	June 12	June 12	June 30	July 6	July 16
Hybrid 143	4513	June 18	June 21	June 23	June 27	June 27	July 6	July 16	July 22
Hybrid 63		June 19	June 21	June 24	do.	do.	do.	do.	July 2
Jenkin.		June 21	do.	do.	do.	do.	do.	do.	July 2
Marquis.	4158	June 13	June 13	June 17	June 21	June 22	June 25	June 30	July 2
Galgalos 39		June 13	June 12	June 8	June 11	June 11	June 27	July 1	July 2
Baart.	1697			May 28	June 2	June 8	June 18	June 22	June 27
Hard Federation.	4733			May 28	June 2	June 8	June 18	June 22	June 27
Plant height (inches).									
Hybrid 128	4512	36	28	14	26	17	13	13	13
Kharof	8249	35	32	30	26	27	19	14	14
Kanad	5146	33	31	31	27	22	14	14	14
Turkey	7366	36	33	30	27	22	14	14	14
Pacific Bluestem.	4067	44	40	39	39	34	32	26	19
Federation	4513	36	32	31	30	26	26	22	19
Hybrid 143	4734	37	36	33	35	33	29	24	19
Federation.		35	29	28	27	22	22	21	19
Hybrid 63		38	33	32	31	30	28	23	21
Jenkin.		35	37	37	34	34	32	27	27
Marquis.	4158	38	36	35	31	33	28	24	24
Galgalos 39		35	35	35	31	33	28	24	24

	1697	36	39	37	35	29
	4733	31	33	32	31	27
Baart						
Hard Federation						
Bushels per acre:						
Hybrid 128						
Khar'kof						
Kanred						
Turkey						
Pacific Bluestem						
Federation						
Hybrid 143						
Hybrid 63						
Jenkin						
Narquis						
Galgates 39						
Baart						
Hard Federation						
Weight per bushel (pounds)						
Hybrid 128						
Khar'kof						
Kanred						
Turkey						
Pacific Bluestem						
Federation						
Hybrid 143						
Hybrid 63						
Jenkin						
Narquis						
Galgates 39						
Baart						
Hard Federation						

None or only a very few green heads Sept. 10.

The emergence dates may have varied 1 or 2 days from the averages given.

TABLE 4.—Dates first and fully headed, plant height, yield, and bushel weight of 16 varieties of wheat seeded in nursery rows at intervals during the season of 1925-26 at Moro, Oreg.

Item and variety	C. L. No.	Date sown (first) and date emerged " (second)															
		Oct 14	Oct 26	Nov. 13 <sup>b</sup>	Dec. 17	Jan. 25 <sup>b</sup>	Feb. 8	Feb. 15	Feb. 22	Mar. 1	Mar. 6	Mar. 18	Apr. 3	Apr. 16	Apr. 30	May 21	June 4
Date first headed:																	
Hybrid 128.	4512	May 14	May 26	May 23	June 2	June 6	June 12	June 23	July 6	June 22	(c)	June 25	(c)	(c)	(c)	(c)	(c)
Kharok.	8249	May 15	May 23	May 23	June 3	June 3	June 6	June 9	June 16	June 23	(c)	June 25	(c)	(c)	(c)	(c)	(c)
Oro.	8220	May 13	June 1	June 1	June 10	June 10	June 13	June 17	June 21	June 24	June 24	June 26	June 26	June 26	June 26	June 26	June 26
Ridit.	6703	May 13	June 1	June 1	June 10	June 10	June 13	June 17	June 21	June 24	June 24	June 26	June 26	June 26	June 26	June 26	June 26
White Odessa.	4655	May 12	June 1	June 1	June 6	June 6	June 8	June 8	June 9	June 9	June 11	June 11	June 11	June 11	June 11	June 11	June 11
Fortyfold	4156	May 18	June 1	June 1	June 6	June 6	June 8	June 8	June 9	June 9	June 11	June 11	June 11	June 11	June 11	June 11	June 11
Triplet.	5408	May 14	June 1	June 1	June 6	June 6	June 8	June 8	June 9	June 9	June 11	June 11	June 11	June 11	June 11	June 11	June 11
Regal.	7364	May 14	June 1	June 1	June 6	June 6	June 8	June 8	June 9	June 9	June 11	June 11	June 11	June 11	June 11	June 11	June 11
Kaured.	5146	May 23	May 31	May 31	June 3	June 3	June 3	June 4	June 4	June 4	June 4	June 4	June 4	June 4	June 4	June 4	June 4
Blackhall.	6251	May 15	May 22	May 22	June 3	June 3	June 3	June 4	June 4	June 4	June 4	June 4	June 4	June 4	June 4	June 4	June 4
Turkey.	7366	May 22	May 26	May 26	June 3	June 3	June 3	June 4	June 4	June 4	June 4	June 4	June 4	June 4	June 4	June 4	June 4
Pacific Bluestem.	4067	May 17	May 22	May 22	June 3	June 3	June 3	June 4	June 4	June 4	June 4	June 4	June 4	June 4	June 4	June 4	June 4
Federation.	4734	May 11	May 13	May 13	May 17	May 17	May 21	May 21	May 24	May 24	May 25	May 25	May 25	May 25	May 25	May 25	May 25
Hybrid 63.	4158	May 11	May 23	May 23	May 30	June 3	June 2	June 3	June 3	June 3	June 3	June 3	June 3	June 3	June 3	June 3	June 3
Marquis.	4158	May 11	May 23	May 23	May 30	June 3	June 2	June 3	June 3	June 3	June 3	June 3	June 3	June 3	June 3	June 3	June 3
Hard Federation	4733	May 4	May 4	May 4	May 10	May 12	May 14	May 16	May 17	May 18	May 19	May 23	June 2	June 8	June 19	July 7	July 31 <sup>a</sup>
Date fully headed:																	
Hybrid 128.	4512	May 27	June 4	June 4	June 8	June 17	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26
Kharok.	8249	May 28	June 2	June 2	June 9	June 15	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16
Oro.	8220	May 28	June 2	June 2	June 9	June 15	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16
Ridit.	6703	May 22	June 9	June 9	June 16	June 23	June 25	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26
White Odessa.	4755	May 28	June 9	June 9	June 16	June 23	June 25	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26
Fortyfold	4156	May 22	June 9	June 9	June 16	June 23	June 25	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26
Triplet.	5408	May 22	June 9	June 9	June 16	June 23	June 25	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26	June 26
Regal.	7364	May 29	June 2	June 2	June 9	June 15	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16
Kaured.	5146	May 29	June 2	June 2	June 9	June 15	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16
Blackhall.	6251	May 22	May 26	May 26	June 2	June 7	June 8	June 8	June 8	June 8	June 8	June 8	June 8	June 8	June 8	June 8	June 8
Turkey.	7366	May 20	May 28	May 28	June 1	June 6	June 7	June 8	June 8	June 8	June 8	June 8	June 8	June 8	June 8	June 8	June 8
Pacific Bluestem.	4067	May 20	May 28	May 28	June 1	June 6	June 7	June 8	June 8	June 8	June 8	June 8	June 8	June 8	June 8	June 8	June 8
Federation.	4734	May 14	May 19	May 19	May 23	May 28	June 1	June 1	June 1	June 1	June 1	June 1	June 1	June 1	June 1	June 1	June 1
Hybrid C3.	4158	May 27	June 2	June 2	June 6	June 13	June 15	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16
Marquis.	4158	May 27	June 2	June 2	June 6	June 13	June 15	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16	June 16
Hard Federation.	4733	May 14	May 19	May 19	May 21	May 21	May 21	May 21	May 21	May 21	May 21	May 21	May 21	May 21	May 21	May 21	May 21
Plant height (inches).																	
Hybrid 128.	4512	35	33	33	33	29	23	18	16	21	19	14	14	14	14	14	14
Kharok.	8249	35	33	33	34	32	29	23	15	21	19	14	14	14	14	14	14
Oro.	8220	35	33	33	34	32	29	23	15	21	19	14	14	14	14	14	14
Ridit.	6703	38	34	34	33	33	29	23	19	21	19	14	14	14	14	14	14
White Odessa.	4655	38	34	34	33	33	29	23	19	21	19	14	14	14	14	14	14

Fortyfold	4136	26	24.3	27.0	24.1	17.9	11.2	30	20	18	16																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
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\* None or only very few green heads, Sept. 10  
 † Very poor stand due dry seed bed, but headed normally.

\* The emergence dates may have varied 1 or 2 days from the averages given  
 † Due to snow covering exact date of emergence not determined.

TABLE 5.—*Dates first and fully headed, plant height, yield, and bushel weight of eight varieties of wheat sowed in nursery rows at intervals during the season of 1926-27 at Moro, Oreg.*

Item and variety	C. I. No	Date sown (first) and date emerged <sup>a</sup> (second)							
		Oct. 20	Feb 15	Feb. 25	Mar. 7	Mar. 14	Mar. 21	Mar 26	Apr. 2
		Nov. 1	Mar 12	Mar. 16	Mar 22	Mar. 31	Apr 8	Apr. 14	Apr. 17
Date first headed:									
Hybrid 128.....	4512	June 5	June 17	June 21	June 30	July 1	( <sup>b</sup> )	( <sup>b</sup> )	( <sup>b</sup> )
Kharkof.....	8249	June 4	do.....	June 17	June 20	June 22	June 28	July 5	July 10
Kanred.....	5146	do.....	June 16	June 18	June 21	June 24	July 1	July 6	July 11
Turkey.....	7366	do.....	June 15	do.....	June 19	June 21	June 27	do.....	July 7
Pacific Bluestem.....	4067	June 15	June 11	June 12	June 15	June 16	June 16	June 17	June 20
Federation.....	4734	June 12	June 6	June 8	June 9	June 10	June 11	June 13	June 16
Marquis.....	4158	June 13	June 11	June 12	June 14	June 15	June 15	June 16	June 18
Hard Federation.....	4733	June 5	May 31	June 2	June 4	June 5	June 6	June 8	June 9
Date fully headed.									
Hybrid 128.....	4512	June 11	June 25	July 16	do.....	do.....	do.....	do.....	do.....
Kharkof.....	8249	June 10	June 24	June 24	July 6	July 11	July 17	July 24	Aug. 1
Kanred.....	5146	do.....	June 21	do.....	July 3	July 8	July 18	July 25	Aug. 2
Turkey.....	7366	June 11	June 19	June 21	June 24	July 5	July 8	July 21	July 27
Pacific Bluestem.....	4067	June 21	June 18	June 19	June 20	June 21	June 21	June 22	June 29
Federation.....	4734	June 18	June 12	June 16	June 18	June 18	June 18	June 19	June 22
Marquis.....	4158	do.....	June 18	June 19	June 19	June 19	June 19	June 20	June 23
Hard Federation.....	4733	June 13	June 8	June 10	June 12	June 13	June 14	June 15	June 16
Plant height (inches).									
Hybrid 128.....	4512	36	34	30	22	17	do.....	do.....	do.....
Kharkof.....	8249	33	32	30	29	32	31	25	22
Kanred.....	5146	34	33	25	31	30	31	31	26
Turkey.....	7366	36	34	29	32	30	31	30	26
Pacific Bluestem.....	4067	34	39	33	38	39	37	36	37
Federation.....	4734	25	32	27	22	31	31	26	27
Marquis.....	4158	31	32	32	31	36	34	31	34
Hard Federation.....	4733	26	28	24	25	27	25	25	28
Bushels per acre.									
Hybrid 128.....	4512	33.2	32.7	29.1	10.5	9.1	0	0	0
Kharkof.....	8249	30.1	25.4	32.8	33.4	33.4	22.6	16.1	7.8
Kanred.....	5146	22.0	24.1	21.1	26.8	28.8	22.9	20.5	11.7
Turkey.....	7366	30.1	28.1	32.6	27.0	26.0	23.7	21.6	20.9
Pacific Bluestem.....	4067	27.0	29.2	27.8	32.7	35.7	29.7	23.8	20.0
Federation.....	4734	26.6	37.9	31.7	38.9	40.7	42.1	40.2	37.9
Marquis.....	4158	22.1	31.7	33.1	32.8	34.0	27.1	30.0	30.7
Hard Federation.....	4733	20.8	32.6	23.0	35.3	34.8	31.0	30.4	35.5
Weight per bushel (pounds):									
Hybrid 128.....	4512	60.5	54.0	do.....	do.....	do.....	do.....	do.....	do.....
Kharkof.....	8249	63.0	do.....	do.....	do.....	do.....	do.....	do.....	do.....
Kanred.....	5146	61.5	56.0	62.0	do.....	58.0	55.0	58.0	do.....
Turkey.....	7366	62.0	55.0	56.5	57.0	55.0	52.0	56.0	56.5
Pacific Bluestem.....	4067	60.0	54.5	60.0	54.5	56.0	58.0	59.0	55.0
Federation.....	4734	60.0	56.0	60.0	59.0	60.5	59.0	60.0	59.0
Marquis.....	4158	60.5	58.0	60.5	57.0	59.0	58.0	58.0	58.0
Hard Federation.....	4733	61.0	61.0	61.5	61.0	61.0	61.0	58.0	61.0

<sup>a</sup> The emergence dates may have varied 1 or 2 days from the averages given.<sup>b</sup> None or only very few green heads Sept. 10.

TABLE 5.—*Dates first and fully headed, plant height, yield, and bushel weight of eight varieties of wheat seeded in nursery rows at intervals during the season of 1926-27 at Moro, Oreg.—Continued*

Item and variety	Date sown (first) and date emerged <sup>a</sup> (second)								
	Apr. 9 Apr. 19	Apr. 16 Apr. 25	Apr. 23 May 2	Apr. 30 May 7	May 7 May 15	May 15 May 22	May 23 May 29	May 31 June 6	July 6 July 11
Date first headed.									
Hybrid 128.....	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	-----
Kharkof.....	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	-----
Kanred.....	July 19	(b)	(b)	(b)	(b)	(b)	(b)	(b)	-----
Turkey.....	July 11	July 19	(b)	(b)	(b)	(b)	(b)	(b)	-----
Pacific Bluestem.....	June 21	July 2	July 7	July 11	July 14	July 22	July 28	(b)	-----
Federation.....	June 19	June 23	June 30	July 5	July 10	July 19	July 25	Aug. 3	-----
Marquis.....	do	June 21	June 24	July 1	July 5	July 11	July 18	July 22	Aug. 27
Hard Federation.....	June 11	June 14	June 15	June 20	June 24	July 2	July 9	July 14	Aug. 18
Date fully headed.									
Hybrid 128.....	-----	-----	-----	-----	-----	-----	-----	-----	-----
Kharkof.....	-----	-----	-----	-----	-----	-----	-----	-----	-----
Kanred.....	-----	-----	-----	-----	-----	-----	-----	-----	-----
Turkey.....	July 20	Aug. 4	-----	-----	-----	-----	-----	-----	-----
Pacific Bluestem.....	July 3	July 10	July 12	July 17	July 22	Aug. 2	-----	-----	-----
Federation.....	June 28	July 3	July 8	July 13	July 28	Aug. 3	-----	-----	-----
Marquis.....	June 27	June 30	July 3	July 10	July 11	July 17	July 22	July 26	Sept. 15
Hard Federation.....	June 18	June 18	June 21	June 28	July 3	July 11	July 19	July 24	Sept. 7
Plant height (inches):									
Hybrid 128.....	-----	-----	-----	-----	-----	-----	-----	-----	-----
Kharkof.....	-----	-----	-----	-----	-----	-----	-----	-----	-----
Kanred.....	18	-----	-----	-----	-----	-----	-----	-----	-----
Turkey.....	23	22	-----	-----	-----	-----	-----	-----	-----
Pacific Bluestem.....	39	36	36	29	27	22	17	-----	-----
Federation.....	29	29	34	28	27	22	17	14	-----
Marquis.....	34	33	35	34	30	26	23	20	-----
Hard Federation.....	31	26	28	28	26	23	23	20	-----
Bushels per acre.									
Hybrid 128.....	0	0	0	0	0	0	0	0	-----
Kharkof.....	0	0	0	0	0	0	0	0	-----
Kanred.....	8.3	0	0	0	0	0	0	0	-----
Turkey.....	16.5	5.9	0	0	0	0	0	0	-----
Pacific Bluestem.....	26.7	30.0	15.5	19.4	16.6	15.7	15.7	0	-----
Federation.....	35.8	39.1	24.9	18.9	16.5	12.5	13.4	11.4	-----
Marquis.....	27.6	27.5	17.9	13.8	16.8	17.9	22.1	12.2	-----
Hard Federation.....	33.1	34.8	27.5	22.7	25.6	18.1	16.3	11.8	-----
Weight per bushel (pounds).									
Hybrid 128.....	-----	-----	-----	-----	-----	-----	-----	-----	-----
Kharkof.....	-----	-----	-----	-----	-----	-----	-----	-----	-----
Kanred.....	-----	-----	-----	-----	-----	-----	-----	-----	-----
Turkey.....	-----	-----	-----	-----	-----	-----	-----	-----	-----
Pacific Bluestem.....	53.5	57.0	48.5	-----	-----	-----	-----	-----	-----
Federation.....	54.0	58.0	49.0	-----	-----	-----	-----	-----	-----
Marquis.....	58.0	60.5	54.0	-----	-----	-----	-----	-----	-----
Hard Federation.....	59.0	60.0	59.0	-----	-----	-----	-----	-----	-----

<sup>a</sup> The emergence dates may have varied 1 or 2 days from the averages given.<sup>b</sup> None or only very few green heads Sept. 10.

## EFFECT OF SEASON ON DATE OF HEADING AND YIELD

In these experiments for all winter varieties there was a critical date for sowing, the varieties if sown after that date producing few or no heads during the same season. This is especially well illustrated by Hybrid 128, which did not head when sown after about February 15. There is, however, some variation in this critical date from season to season. Thus, Hybrid 128 did not head normally in 1926 when sown after February 8, while in 1927 it headed normally when sown as late as February 25. The critical date for sowing Hybrid 128 was 9 days later in 1925 and 20 days later in 1927 than in 1926. The differences were even greater for other varieties. This variation from season to season apparently is correlated with differences in temperature, amount of sunshine, and other factors, the combined effect of which is to produce an internal condition in the plant, caus-

Federation and Pacific Bluestem heretofore have been considered true spring varieties. In these experiments they did not head when sown on May 21 and on June 4 in 1926, but produced only a flat vegetative growth. These varieties therefore should be considered as intermediate. They carry a factor for winter-growth habit, but their critical sowing dates are later than the normal date for seeding spring wheat in Oregon.

The true spring varieties, Marquis and Hard Federation, produce heads when sown at any time the summer, provided sufficient moisture is present for germination and growth and they are not killed by frost. Volunteer Hard Federation plants have been observed coming into head in November. Hard Federation and Marquis sown on June 4, 1926, were starting to head July 18 and July 31, respectively, 44 and 57 days after seeding. Sunset and Quality, sown July 6, 1927, were first headed August 10 and fully headed August 29 and September 9, respectively. The low yields of these varieties from late seedings are due partially to poor stands and unfavorable growth conditions caused by high temperatures and dry soil.

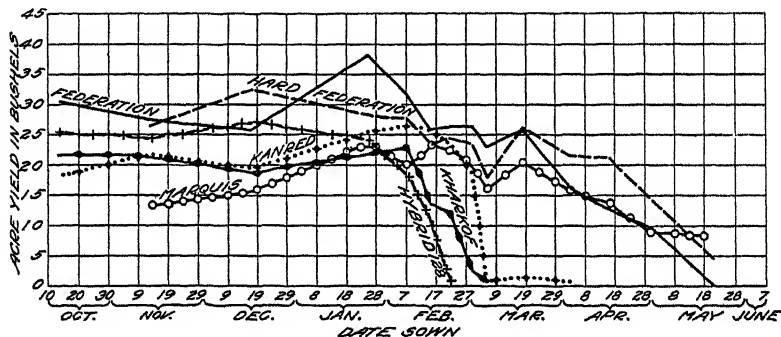


FIGURE 4.—Yield of six varieties of wheat sown at intervals from October 14, 1925, to May 21, 1926, at Moro, Oreg.

In general, wheat varieties head in the same relative order when sown on different dates during the season. Some varieties vary from this tendency. When sown November 13, 1925, Federation headed nine days earlier than Marquis; sown on April 3, 1926, they headed on the same day; and sown on April 30, Marquis headed six days earlier. Sown on May 21, Federation produced only a prostrate vegetative growth and did not head, whereas Marquis headed normally. The reversal is well shown in Figure 3. The same reversal also occurred in 1926 and 1927, although the dates when the change took place varied with the season. Marquis varied less in heading date when the sowing date was varied than did any of the varieties studied.

The plants were shorter from the later sowings of each variety. The data show no consistent relation between sowing date and weight per bushel, although in general later sowing gave lower test weights.

#### SUMMARY

The data presented show that the date on which wheat will head depends on variety, date of sowing, and seasonal environmental factors.

Every wheat variety which carries a factor for winter-growth habit, if sown after a critical spring date characteristic for that variety, will not head the same season but will produce a prostrate excessive vegetative growth. If not injured by drought or winterkilled, it will head the second season at about the normal date.

There is a wide variation in date of heading between varieties seeded on the same date.

Varieties headed in the same relative order for comparable seeding dates during different seasons, although the actual heading dates varied with the season.

Some varieties varied in relative order of heading for different seeding dates during the same season.

The critical sowing date for yield is earlier than the critical date for heading.

The critical planting date for normal heading at Moro, Oreg., was as early as February 15, for Hybrid 128. This critical date for Federation was not reached until April 30. The varieties studied showed a range from Hybrid 128, with the earliest critical date, to Marquis and Hard Federation, which apparently have none but will head when sown at any time if moisture and temperature are favorable for growth.

The critical dates are earlier in those seasons when the growing season is early and when temperatures are higher than in seasons when the reverse is true.

The growth phases of wheat, such as jointing and heading, are not completely controlled by any one specific environmental factor, such as temperature or the relative length of day, but are influenced by the entire environmental complex.

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# THE VITAMIN A CONTENT OF OATS<sup>1</sup>

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## INTRODUCTION

In 1917, McCollum<sup>2</sup> designated fat-soluble vitamin A, along with proteins and inorganic salts, as a dietary deficiency of the oat kernel. He says:

The unidentified dietary factor fat-soluble A is present in very small amount in the oat kernel. It is not possible to supplement the oat kernel with inorganic salts and purified protein so as to induce growth beyond the third month. The inclusion of butter fat or some other substance which supplies the unknown A prevents the failure at this point \* \* \*.

This finding of McCollum was confirmed in 1927 by Steenbock and Coward,<sup>3</sup> who irradiated the basal food to supply vitamin D. They found that when oats, as the sole source of vitamin A, made up 68 per cent of the basal synthetic ration, ophthalmia developed in rats in 5½ to 6½ weeks, and 3 of the 4 animals died shortly after that time.

Since Sure<sup>4</sup> had found that vitamin A could be detected in wheat oil when the wheat itself appeared to be lacking in this factor, and Meyer and Hetler<sup>5</sup> had demonstrated a slight concentration of vitamin A in the crude oil pressed from corn germs, it was thought possible that some vitamin A might be detected in the fat of the oat kernel. Accordingly, oat oil as well as the oat kernel was tested for this vitamin.

## EXPERIMENTAL PROCEDURE

The curative method for the quantitative determination of vitamin A as described in detail by Meyer and Hetler was used throughout this study. Rats were employed as the experimental animals. The experimental period was started when the rats were 28 to 30 days of age and weighed approximately 50 grams. A vitamin-A-free irradiated basal diet, supplemented with dried brewer's yeast (fifteenthths gram daily) to furnish vitamins B and G, was given the test animals, and they were allowed access to the diet at all times. The basal diet was prepared as formerly described,<sup>6</sup> and was composed of the following ingredients:

	Per cent		Per cent
Extracted casein-----	18	Starch-----	57
Osborne-Mendel salt mixture---	4	Sodium chloride-----	1
Crisco-----	20		

<sup>1</sup> Received for publication Nov. 3, 1930; issued April, 1931.

<sup>2</sup> MCCOLLUM, E. V., SIMMONDS, N., and PRIZ, W. THE NATURE OF THE DIETARY DEFICIENCIES OF THE OAT KERNEL. *Jour. Biol. Chem.* 29: 341-354, illus. 1917.

<sup>3</sup> STEENBOCK, H., and COWARD, K. H. FAT-SOLUBLE VITAMINS. XXVII. THE QUANTITATIVE DETERMINATION OF VITAMIN A. *Jour. Biol. Chem.* 72: 765-779, illus. 1927.

<sup>4</sup> SURE, B. DIETARY REQUIREMENTS FOR FERTILITY AND LACTATION. THE VITAMIN A CONTENT OF WHEAT OIL. *Jour. Agr. Research* 37: 93-99, illus. 1928.

<sup>5</sup> MEYER, C. R., and HETLER, R. A. THE DISTRIBUTION OF VITAMIN A IN SOME CORN-MILLING PRODUCTS. *Jour. Agr. Research* 39: 767-780, illus. 1929.

<sup>6</sup> MEYER, C. R., and HETLER, R. A. *Op. cit.*

With the depleted animals used (from mother rats receiving a diet low in vitamin A) definite eye symptoms usually preceded cessation of growth and developed from 25 to 35 days after the experimental period began. As soon as definite ophthalmia developed, ground whole hull-less oats as the sole source of vitamin A replaced the same percentage of starch or Crisco, or parts of both the starch and the Crisco, in the diet. Later work included the use of oat oil incorporated in the diet or fed separately, as the sole source of vitamin A. The test period was eight weeks, except in the cases where death occurred before the end of that time.

A variety of naked oats, Illinois Hull-less, was used exclusively in this investigation. The oat oil was prepared by a method essentially the same as that used by Sure<sup>7</sup> in the preparation of acetone-extracted wheat-germ oil. The ground whole oats were dried in shallow pans at a depth of about one-half inch in vacuo at 50° C. for 10 hours. About 500 grams of the dried oats were placed in flasks, covered with cold acetone, thoroughly shaken for several minutes, and allowed to stand 10 to 12 hours. The solvent was then filtered off and the oats were further extracted by percolating with cold acetone until no color was visible in the solvent. The combined extracts were dried in the air at room temperature to remove all but the last portion of the acetone, which was completely removed by drying in vacuo at 35° for 3½ hours. It was found that by this method about 3.75 per cent by weight of oil could be extracted from the oats. Munro and Rae<sup>8</sup> state that fresh oats contain about 4.8 per cent oil. The oil was greenish brown in color and readily hardened when placed in the refrigerator, where it was always stored.

When oat oil was incorporated in the food only one or two days' supply of the ration was made up at a time, and the usual procedure of irradiating the basal food before the addition of the test material was followed. Fresh food was given to the animals daily. When the oat oil was fed separately it was found that by placing the yeast on top of the oil, the latter was readily consumed along with the yeast.

A negative control animal was maintained in each litter on the vitamin-A-free basal diet alone. Cod-liver oil, 8 drops daily, was fed to other control animals which had developed severe ophthalmia. In order to make sure that the oat oil was not toxic and that the arrested growth and decline of the animals receiving the oat oil were due solely to vitamin A deficiency, cod-liver oil was given along with the oat oil to one or two animals in each group, in every case after the animal had reached a critical condition.

The potency of the vitamin A in the experimental material was determined by comparing the test animals and the controls in respect to eye condition and growth.

## EXPERIMENTAL RESULTS

The data submitted in Table 1 and Figure 1 show clearly that whole hull-less oats are almost if not totally lacking in vitamin A. Although the experimental animals continued to grow for some time after the

<sup>7</sup> SURE, B. DIETARY REQUIREMENTS FOR REPRODUCTION. V. THE RÔLE OF VARIOUS VEGETABLES AND FRUIT OILS IN FERTILITY AND LACTATION. *Jour. Biol. Chem.* 69: 29-40. 1926.

<sup>8</sup> MUNRO, L. A., and RAE, W. J. DETERMINATION OF THE ANTHRACHITIC PROPERTIES OF OAT OIL. *Sci. Agr.* 10: 305-312, illus. 1930.

development of ophthalmia and following the inclusion of oats in the diet, the ophthalmic condition was never alleviated, and became increasingly more severe. In every case where diets including the 10, 30, or 60 per cent levels of oats were fed, growth finally ceased and a rapid decline in weight followed. Death occurred before the end of the 8-week test period. All negative control rats, or animals receiving the basal diet alone, died about 30 days after the development of ophthalmia. When 8 drops daily of cod-liver oil were given

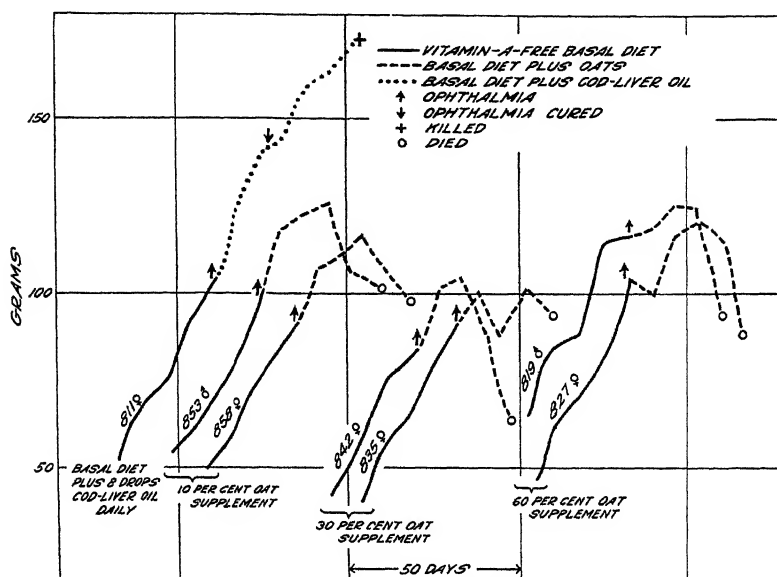


FIGURE 1.—Weight curves of rats receiving vitamin-A-free diets supplemented with different percentages of whole hull-less oats. Ophthalmia was never cured and death resulted in every case. The weight curve of an animal with ophthalmia cured by cod-liver oil is included

to animals in which ophthalmia had developed while they were on a vitamin-A-free basal diet, the eyes were completely cured within a few days. If the eye condition was allowed to become very severe, however, a period as long as two weeks was necessary in order to effect a permanent cure with cod-liver oil. Tabulated data of control animals are not included here, but the results were very similar to those presented in the data for control animals by Meyer and Hetler<sup>9</sup> in 1929.

TABLE 1.—The effect of whole hull-less oats on ophthalmia and growth of vitamin A deficient rats

Rats used	Quantity of oats fed		Effect on ophthalmia	Effect on growth
	Per cent of the diet	Average weight fed daily <sup>1</sup>		
Number		Grams		
5	10	0.6-0.8	Not cured.....	Decline and death in 30-33 days.
6	30	1.6-2.4	do.....	Decline and death in 25-33 days.
6	60	2.4-5	do.....	Decline and death in 17-35 days.

<sup>1</sup> Calculated from total daily food consumption.

<sup>9</sup> MEYER, C. R., and HETLER, R. A. Op. cit.

When the acetone-extracted oat oil was included as a possible source of vitamin A in the diet of ophthalmic rats, the condition of the animals

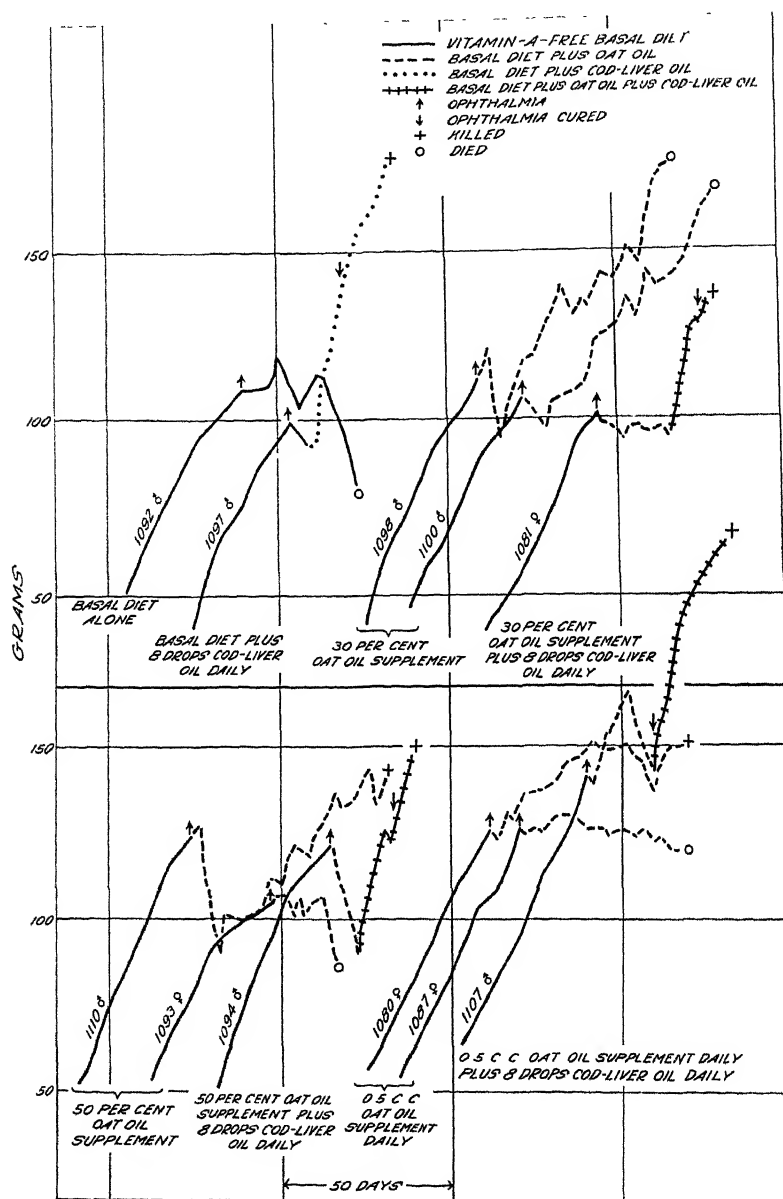


FIGURE 2.—Weight curves of rats receiving vitamin-A-free diets supplemented with oat oil alone, and with both oat oil and cod-liver oil. When the vitamin-A-free basal diet was supplemented by oat oil alone ophthalmia was never cured, growth was arrested, and several animals died. When cod-liver oil was fed with the oat oil, ophthalmia was cured and an excellent nutritive condition was attained. Weight curves of control animals not receiving oat oil are included.

was slightly better than that of rats receiving whole hull-less oats as the sole source of vitamin A. Table 2 and Figure 2 show the results

obtained when the oat oil was fed at different levels in the diet. Since the acetone extraction gave about 3.75 per cent oil by weight from the oats, 1 gram of oat oil represents the acetone-soluble portion of about 27 grams of oats. The oat oil was not only incorporated in the diet at 50 and 30 per cent levels but was also fed separately as 0.5 cubic centimeter daily. The animals receiving 50 per cent oat oil in the diet consumed less food, and thus actually obtained approximately the same amount of oat oil daily as the animals receiving only 30 per cent oat oil in the diet. When cod-liver oil was given to animals in each of these groups, however, the food intake of the rats increased, and in the cases where cod-liver oil was fed along with the 50 per cent oat oil an average of 4 grams daily of the oat oil was consumed. The complete cure of ophthalmia in 7 to 14 days and the excellent nutritive condition of these rats indicate that the poor condition of all of the animals receiving oat oil as the sole source of vitamin A was not due to a toxic effect of the oat oil, but to a deficiency of vitamin A.

TABLE 2.—*The effect of oat oil on ophthalmia and growth of vitamin A deficient rats*

Rats used	Quantity of oat oil fed		Effect on ophthalmia	Effect on growth
	Per cent of the diet	Average weight fed daily		
Number	50	Grams		
8		<sup>1</sup> 1.6	Not cured, several animals showed slight improvement.	Slow or arrested growth, 1 animal died.
2	50	<sup>1</sup> 2.4	Cured with cod-liver oil.....	Decline; death imminent; cured with cod-liver oil.
10	30	<sup>1</sup> 1.6	Not cured, several animals somewhat improved.	Grew slowly, no deaths.
1	30	<sup>1</sup> 1.8	Cured with cod-liver oil.....	Cessation of growth; almost dead; completely cured with cod-liver oil.
10	<sup>1</sup> 5	<sup>1</sup> 3.8	Not cured, severe ophthalmia.....	Arrested growth; decline; 1 animal died.
1	<sup>1</sup> 3.7	<sup>1</sup> 3.8	Cured with cod-liver oil.....	Almost dead; completely cured with cod-liver oil.

<sup>1</sup> Calculated from total daily food consumption.

<sup>2</sup> With 8 drops of cod-liver oil, given daily.

<sup>3</sup> Fed separately as 1 cubic centimeter (or 0.38 gram) daily.

The slow growth and prolongation of life (except in one case, Table 2) over an 8-week period, together with the slight improvement although never permanent cure of ophthalmia in rats fed the larger amounts of oat oil, probably indicate the presence of a very small amount of vitamin A in the oat oil. The quantity present was little more than a trace, however, since it could be detected only when the oat oil was fed at 30 or 50 per cent levels, or approximately 1.6 grams daily. 1.6 grams represents the fat-soluble portion of approximately 43 grams of whole oats, or an amount of oats far too large for a rat to consume daily.

A very recent report by Munro and Rae<sup>10</sup> describes tests for vitamin D in oat oil. These investigators used chickens as the experimental animals, and studied the antirachitic properties of irradiated and nonirradiated oat oil which had been prepared on a large scale

<sup>10</sup> MUNRO, L. A., and RAE, W. J. *Op. cit.*

in their laboratory. The solvent used was a low-boiling fraction from high-test gasoline. The results showed that the oat oil was of no value as an antirachitic agent either before or after irradiation. It appears, therefore, that oat oil is of little if any value as a source of either vitamin D or vitamin A.

#### SUMMARY

An investigation was made of the vitamin A content of whole hull-less oats and of the acetone-extracted oil of whole hull-less oats. Rats were used as the experimental animals. Ophthalmia was not cured and death occurred when whole oats at levels of 10, 30, or 60 per cent of the diet, or from 0.6 to 5 grams daily, were fed to vitamin A depleted animals. When oat oil was fed at levels as high as 30 and 50 per cent of the diet, or 1.6 grams daily, to vitamin A deficient rats, ophthalmia was never entirely cured, but a slight improvement of the eye condition was noted in several cases. All but one animal lived throughout the 8-week test period, but growth was very slow or was completely arrested. It was concluded that a very small amount of vitamin A is present in oat oil.

The rapid cure of ophthalmia and the resumption of growth which occurred when cod-liver oil was added to the diet of rats that were declining on a diet in which oat oil furnished the sole source of vitamin A, show that the poor condition of these animals was not due to a toxic effect of the supplement but to a deficiency of vitamin A in the oat oil.

# INHERITANCE IN A WHEAT CROSS BETWEEN HYBRID 128 × WHITE ODESSA AND KANRED<sup>1</sup>

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## INTRODUCTION

The genetic data herein presented were obtained from a wheat cross which was made primarily to develop a variety of wheat resistant to bunt, *Tilletia tritici* (Bjerk.) Wint. The parents used were a homozygous bunt-resistant strain obtained from the Washington experiment station, Hybrid 128 × White Odessa, and a pure line of Kanred.

## LITERATURE

Biffen (1)<sup>2</sup> was the first to work on the inheritance of awns in wheat.

The Howards (7) crossed a fully awned wheat with a fully awnless one and found 15 awned : 1 awnless when all degrees of awned plants were grouped together.

Clark and Hooker (4) found in studying the inheritance of awns in Marquis × Hard Federation that a 1 : 12 : 3 ratio was obtained in one case and a 1 : 11 : 4 ratio in another. Hard Federation they classed as awnless and Marquis as awnletted. In the F<sub>2</sub> generation a close approximation to a 3 awnless : 1 awnletted ratio was obtained. If the awnletted and the intermediates were combined, a ratio of 15 awnletted : 1 awnless was obtained. A 2-factor difference is suggested to explain these results. Both parental types were recovered in the F<sub>3</sub> and one true-breeding intermediate class was produced.

Stewart and Heywood (13) in a study of a cross between Federation and F22, a hybrid of Sevier × Dicklow, which were awnless and awned, respectively, report four true-breeding awn classes and five segregating classes in the F<sub>3</sub> progenies. A ratio of 1 : 2 : 2 : 4 : 1 : 2 : 1 : 2 : 1 was suggested and a rather close fit obtained. In this case the awn inheritance was explained on the basis of a 2-factor difference with independent segregation.

When Marquis and Federation wheats were crossed, Stewart and Tingey (14) found a 1-factor difference to explain the breeding behavior. In this case both parents were almost entirely awnless except for short apical beaks, and the F<sub>3</sub> progeny were either like the parents or bred true for apical awns or segregated for all three classes in a 1 : 2 : 1 ratio.

Gaines and Singleton (5) found in a Turkey × Marquis cross that when the fully awned F<sub>3</sub> progenies were compared with the remainder of the progenies, a 3 : 1 ratio was obtained for awnless and awned, respectively. If the heterozygous were considered a separate class from the pure dominant a 1 : 2 : 1 ratio resulted.

<sup>1</sup> Received for publication Nov. 3, 1930; issued April, 1931. Contribution from the department of agronomy, Utah Agricultural Experiment Station.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 520.



Clark, Florell, and Hooker (3) report crosses between Bobs, Hard Federation, and Propo in which five true-breeding awn classes were obtained. In this case the awnless condition was considered dominant, although there is no known record of real dominance for awn characters. Their results led them to suggest two major factors *AABB* for Bobs  $\times$  Propo, two minor factors for Bobs  $\times$  Hard Federation, and two major factors and one minor factor for Hard Federation  $\times$  Propo which involved the five true-breeding awn classes. They concluded that from one to as many as four genetic factors may be involved in the inheritance of awnedness in wheat.

Parker (9), in a cross between a club and an ordinary wheat of intermediate density, obtained forms as compact as the more compact parent and a series of less dense forms, some of which were more lax than the more lax parent.

Stewart (12) found in a cross of Kanred  $\times$  Sevier that the  $F_1$  plants were intermediate for spike density but resembled the Sevier parent more closely than the Kanred. In the  $F_2$ , lines were found more dense than Sevier and more lax than Kanred. No true-breeding lines like the Sevier parent were recovered. The intermediates were all heterozygous, and a close fit to a 1:2:1 ratio was found, which agrees with Spillman's (10) data.

Stewart and Heywood (13) obtained the same results in a cross of Federation  $\times$  a hybrid of Sevier  $\times$  Dicklow. In both cases, the classification of spike types was based on measurements of a representative spike of each plant. The mean coefficients of variability for the spike density of parental rows were 9.81 per cent and 10.59 per cent, while for the  $F_2$  lax progenies the mean coefficient of variability was 9.56 per cent and for the homozygous-dense progenies 10.70 per cent. The heterozygous rows showed a mean of 40.91 per cent, which makes the divisions rather clear-cut. A close approximation to the expected 1:2:1 ratio was obtained, indicating a single-factor difference in the parents.

Nilsson-Ehle, cited by Stewart (11), reports the presence of two lengthening factors in some crosses in order to explain the behavior of spike density.

The dominance of red kernel over white was first reported by Biffen (1). He found that in the  $F_1$  generation all kernels were red, and in the  $F_2$  there were 3 red : 1 white. Nilsson-Ehle first reported crosses in which ratios of 15 red : 1 white and 63 red : 1 white were obtained in the  $F_2$  segregates. Clark (2) also found that red color is dominant, and that either one, two, or three factor differences may occur between crosses of various wheat varieties for this character. In the  $F_2$  he obtained 95.1 per cent red and 4.9 per cent white, which if corrected on a 2-factor-difference basis, would be 93.75 per cent and 6.25 per cent, respectively. When he used Kota in a reciprocal cross as the female, a closer fit was obtained. In all tests the white bred true, while the red-kernelled plants threw segregates ranging from 63 red : 1 white, 15 red : 1 white, and 3 red : 1 white, depending on the cross.

Stewart (12) found a 3-factor difference in a Kanred  $\times$  Sevier cross. As only from 30 to 40 plants were harvested from a row, the segregating group of 63 red : 1 white fell short of the calculated expectancy. By growing an  $F_4$  series he was able to adjust this error to a great extent, and to settle doubtful cases in other groups as well.

Stewart and Tingey (14) studied the kernel color in a Marquis  $\times$  Federation cross which indicated an apparent 2-factor difference. The closeness of fit in this case was shown by  $P=0.6155$ , which is considered very close.

Love (8) and Hayes (6) report a one, two, or three factor difference in various wheat crosses involving different varieties.

#### DESCRIPTION OF PARENTS

Kanred originated as a pure line from a Crimean wheat at the Kansas experiment station. It is very much like ordinary Turkey, but can be distinguished from it by the longer beaks on its glumes. It has a white glume with faint markings of bronze and a dark-red kernel. Its fusiform spike is a lax to mid-dense, and is fully awned. Kanred has the winter growth habit and is known to be susceptible to several physiologic forms of bunt. At the present time it is the most important wheat in the central Great Plains area, where it out-yields ordinary Turkey and is believed to be a little more drought resistant. In parts of the intermountain region also, Kanred has been reported to outyield Turkey and is now becoming more popular than Turkey as a winter wheat.

Hybrid 128  $\times$  White Odessa was obtained from Gaines of the Washington station. It is a homozygous strain for the characters studied and has white chaff and white kernels. The head type would be classed as club or dense and the upper third of the spike bears tip awns which in this study are classed as awns 2.

The various awn types of wheat have been arbitrarily grouped into four classes: Awns 1, awns 2, awns 3, and awns 4. Class 1 is free from awns except for short beaks and for one or more very short awn tips of the apex of the spike. Class 2 is characterized by awns ranging in length from 4 to over 20 mm. Awns in this class are generally distributed over the upper third of the spike. Class 3 awns are usually distributed over the upper two-thirds of the spike, being longer near the apex. Several short curly awns may be found below the longer ones, causing a wider range of variability to occur in this class than in any other class studied. Awn class 4 is the common fully awned type of which Turkey is a representative variety.

In Table 1 the contrasted characters of the parents studied in this cross are presented.

TABLE 1.—*Parental differences between Hybrid 128  $\times$  White Odessa and Kanred wheats*

Variety	Awns	Spike density	Kernel color	Resistance to bunt smut
Hybrid 128 $\times$ White Odessa...	Apically awned...	Club or dense....	White.....	Highly resistant.
Kanred.....	Fully awned.....	Lax to mid lax....	Dark red....	Susceptible.

#### EXPERIMENTAL PROCEDURE

During the summer of 1926, the cross between a pure line of Hybrid 128  $\times$  White Odessa and a pure line of Kanred was made at Newton, Utah, a few miles from the experiment station. The  $F_1$  generation was grown in 1927 and the  $F_2$  in 1928. One of the most vigorous

families was then chosen to continue the experiment. Segregates in the  $F_2$  were so grouped that the  $F_3$  progenies could be seeded systematically, according to the awn class, starting with awn class 2 followed in order by awn classes 3 and 4.

The  $F_3$  progeny rows were spaced 12 inches apart and the kernels from each  $F_2$  plant were used to seed one row. The kernels were spaced by hand without measuring from  $2\frac{1}{2}$  to 4 inches apart in the rows, making from 40 to 60 plants in a progeny row 12 feet in length. As the primary aim in making the cross was to get a good variety of wheat resistant to bunt, the  $F_2$  kernels were dusted with all the smut spores that would remain on them.

Each  $F_3$  progeny was used to represent the breeding behavior of the  $F_2$  plant. This method has been found much more accurate, as genotypic differences may not clearly manifest themselves in the  $F_2$  but show in the  $F_3$ . Often this method can be improved by again growing  $F_4$  progenies when in doubt of  $F_3$  results.

Too few parental rows were grown along with this cross for accurate comparisons of measured characters, but the data are included.

When the grain was mature, the  $F_3$  rows were pulled separately and labeled, after which they were taken to the laboratory where each plant was carefully classified and measured. The data were taken shortly after harvest in order to permit the fall seeding of the most promising lines.

Owing to the artificial inoculation with smut of the  $F_2$  seed, several  $F_3$  rows contained fewer than 40 plants which had one or more normal spikes (i. e., free from bunt). Occasionally more than one-half of the individuals of a progeny would be completely smutty. When all the spikes of a plant contained bunt, kernel color and spike density could not be determined.

After the data were all taken, tabulated, and analyzed, the inheritance of each character was studied separately and a theory sought to explain the results. The inheritance of awns and kernel color in this cross were clear-cut cases of segregation which could be determined by observation, but with spike density a measurement of 10 internodes was thought to be more accurate. The mean value, the standard deviation, and the coefficient of variability were calculated for the spike density of each  $F_3$  progeny.

#### INHERITANCE OF INDIVIDUAL CHARACTERS

Inheritance studies were made of the following individual characters: Awn classes, spike density, and kernel color.

##### AWN CLASSES

Although the  $F_1$  plants resembled the Hybrid 128  $\times$  White Odessa parent for awn development more closely than they did the Kanred parent, they were intermediate. In the  $F_2$ , both parental types were recovered as well as a group of intermediates which segregated in the  $F_3$ . When the  $F_3$  progenies were studied as a basis for the genotypic classification of the  $F_2$  plants, 60 of the  $F_2$  plants were shown to be homozygous for awns 2; 120 of the  $F_2$  plants were heterozygous, throwing all three groups in the  $F_3$  generation; and 54 plants were homozygous for awns 4. Figure 1 shows typical heads from  $F_3$  plants for each of the classes found in this cross.

The length of the awns was not measured, as rather clear-cut segregation groups could be made by observation. Awns of class 2 seldom reached over 1 cm. in length and were limited to a few on the upper third of the spike. Awns of class 3 were not only considerably longer, but were generally more numerous and extended farther down the spike than did awns of class 2. The behavior of awns of class 3 in this cross was very different from that reported by the Howards (7) of India, who found that such awns are rather short but cover the larger portion of the spike. Awns of class 3, however, showed a greater range of variability than either of the true-breeding groups. The homozygous awns of class 4 showed some variation in length;

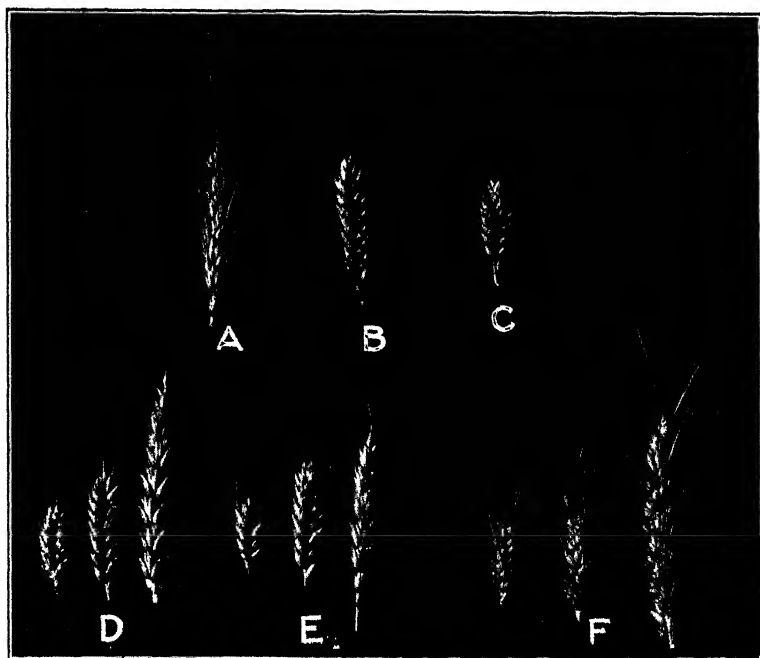


FIGURE 1.—Type of awns found in the three awn classes of Hybrid 128×White Odessa and Kanred wheat, that appeared in the  $F_2$  generation, the two parent types and the  $F_1$  type: A, Kanred parent; B,  $F_1$  type; C, Hybrid 128×White Odessa parent; D, awn class 2; E, awn class 3; F, awn class 4

the lax spikes had longer awns than the dense ones, but in general they were very nearly like Kanred in this character, a behavior previously studied at some length (12).

The four awn classes found in the earlier study (12) were designated as follows:

Awn 4	-----	<i>AABB</i>
Awn 3	-----	<i>AAbb</i>
Awn 2	-----	<i>aaBB</i>
Awn 1	-----	<i>aabb</i>

Table 2 shows the three awn classes based on their genotypic differences, their expected ratio, and their breeding behavior on the assumption that a 1-factor difference is present, as the data of this cross seem to indicate.

TABLE 2.—Three awn-class genotypes, their expected ratios, and their breeding behavior based on a 1-factor difference

Awn-class No	Expected ratio	Genotype	Expected breeding behavior
4.....	1	.1.1BB	Breed true for awn class 4.
3.....	2	.1aBB	Segregates for awns 2, 3, and 4.
2.....	1	aaBB	Breed true for awn class 2.

Table 3 shows the goodness of fit between the observed and calculated frequencies. On the basis of three awn classes,  $\chi^2=0.4614$  and  $P=0.8190$ , which is a very good fit. A worse fit would be expected in 82 out of each 100 cases due to chance alone.

TABLE 3.—Goodness of fit of three awn-genotype classes of  $F_3$  progenies when compared with a 1:2:1 ratio which would be expected theoretically when a 1-factor difference segregates independently

Item	Calculated value (C)	Observed value (O)	O-C	(O-C) <sup>2</sup>	(O-C) <sup>2</sup> / C
Homozygous, awns of class 2.....	58.5	60	1.5	2.25	0.0385
Heterozygous.....	117	120	3	9	.0769
Homozygous, awns of class 4.....	58.5	54	-4.5	20.25	.3461

$$\chi^2=0.4615. \quad P=0.8190.$$

## SPIKE DENSITY

The  $F_2$  breeding behavior for spike density was studied by a measurement of 10 rachis internodes on a leading spike of each  $F_2$  plant. The extreme upper and the extreme lower internodes were avoided as they are more variable than the central ones. The first 40 of the plants were taken at random from each bundle and used to represent that particular  $F_2$  plant which was their parent the previous year.

The homozygous progenies were separated from the heterozygous by a statistical method in which the coefficient of variability was used as the final basis. The parental rows of Kanred showed coefficients of variability ranging from 6 to 16 per cent, with a mean of 9.46 per cent. (Table 4.)

The range in coefficient of variability was not obtained for the Hybrid 128  $\times$  White Odessa parent, as only a single row was grown. This row, however, gave a coefficient of variability of 4.76 per cent. The homozygous-dense progenies showed a range in coefficients of variability of 1.26 to 9.85 per cent, with a mean of 6.29 per cent. The homozygous-lax progenies showed a range of from 3.26 to 11.48 per cent. There was one progeny the coefficient of variability of which was 23.14 per cent. The spike density of this row ranged from 46 to 60 mm. (the ordinary range for true-breeding lax progenies), and contained only 15 plants which were not completely affected with smut. On account of there being no really dense spikes the progeny was regarded as homozygous-lax. However, with as high a percentage of variability as was shown in this exceptional row, it might statistically be thought to overlap on the lowest coefficient of variability of the segregating rows, which is 25.96 per cent. This progeny might have been regarded as segregating, without affecting the conclusions in any way.

TABLE 1.—Mean spike-density and coefficient-of-variability (C. V.) classes of Hybrid 128×White Odessa and Kanred wheat, and of the three groups of hybrid F<sub>3</sub> progenies, homozygous-dense, heterozygous, and homozygous-lax

Parent or progeny	Number of plants in spike density classes (millimeters per 10 internodes) specified																Total	C. V. classes
	18	21	24	27	30	33	36	39	42	45	48	51	54	57				
Kanred.....												2	5	1			2	<i>Per cent</i>
																	6	6.00
																	2	9.00
																	2	12.00
																	2	15.00
Total or mean.....												9	1				10	9.46
Hybrid 128×White Odessa.....		1															1	4.76
Homozygous-dense.....	1	2															3	2.00
	9	13	1														23	4.00
	9	22															31	6.00
	1	6	1														8	8.00
Total or mean.....	20	43	2														65	6.29
Heterozygous.....						1											1	24.00
						1											1	20.00
					1	2											4	28.00
					3	2	4	2									11	30.00
						6	11	1									18	32.00
					2	10	11	2									25	34.00
					1	6	16	7	1								31	36.00
					2	6	4	1									13	38.00
					4	2											6	40.00
						1											1	42.00
Total or mean.....			1	18	45	40	7										111	31.95
Homozygous-lax.....										1	11	10					22	2.00
										3	6	16					29	5.00
											1	2					5	8.00
													2				2	11.00
													1				1	14.00
																		17.00
																	1	23.00
Total or mean.....										4	18	29	7				58	5.89

The heterozygous progenies had a coefficient of variability ranging from 26 to 42 per cent with a mean of 31.95 per cent. Only a few of the progenies showed less variability than 30 or greater than 35 per cent.

The coefficient-of-variability method of spike-density classification showed clear-cut separation of the three groups found in this cross with one exception mentioned previously, which falls close to the lower limits of the heterozygous expectancy.

The mean values for the coefficient of variability of both homozygous progeny groups were lower than the mean for Kanred and higher than the mean for Hybrid 128×White Odessa. Less variability, as shown by lower coefficients of variability, was obtained in the homozygous-dense group than in the homozygous-lax group. With one exception the coefficient of variability of the least variable heterozygous progeny exceeded the most variable of the homozygous groups by over 100 per cent.

Spikes and rachises from parents and progeny of the cross are shown in Figure 2, and spike-density curves are shown in Figure 3.

When the breeding behavior of the  $F_3$  progenies was analyzed, there were found to be 65 homozygous for dense spikes, 111 heterozygous, and 58 homozygous for lax spikes. Since a complete  $F_3$  progeny repre-

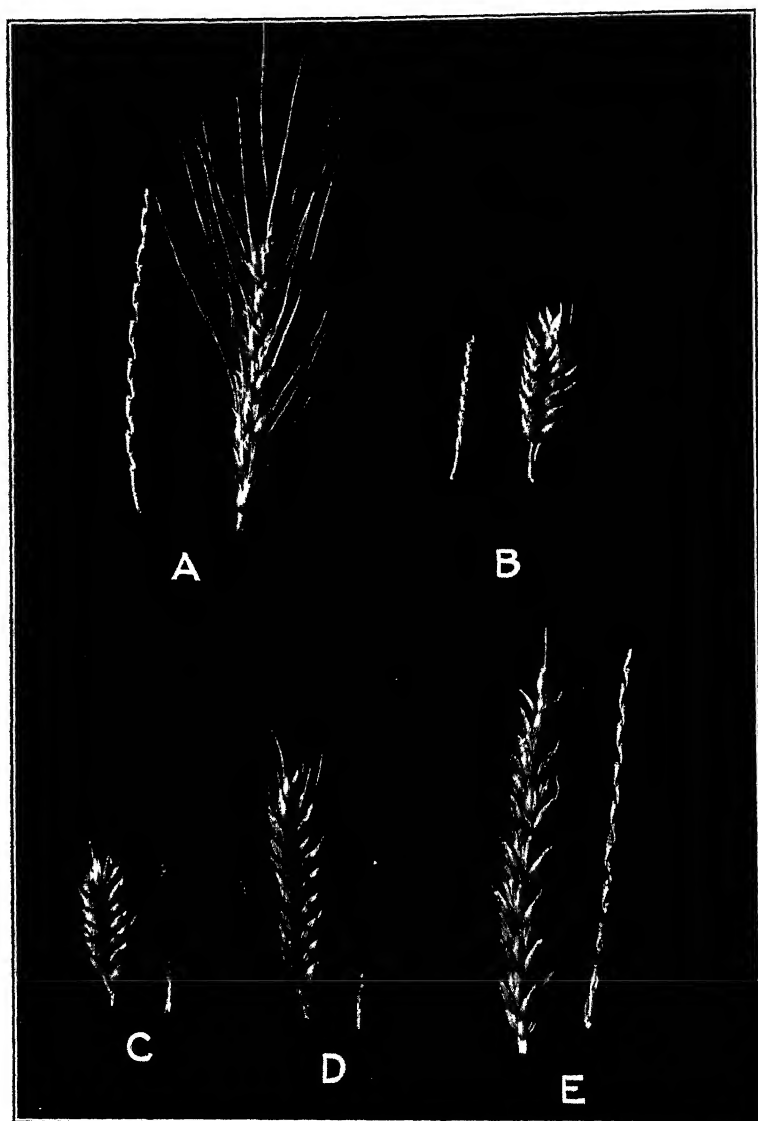


FIGURE 2.—Spikes and rachises from parents and progeny of the Kanred cross with Hybrid 128X White Odessa. A, Kanred parent; B, Hybrid 128X White Odessa parent; C, representative spikes and rachis of homozygous-dense progeny; D, typical spike and rachis of intermediate progeny group; E, spike and rachis of typical homozygous-lax  $F_3$  progenies. Rather wide variation occurred in each  $F_3$  spike-density group

sents an  $F_2$  plant, the  $F_3$  progeny gives a more accurate description of the  $F_2$  plant than could be obtained by trying to determine the possible genetic composition of the plant itself. Both parental types were

recovered in the homozygous progenies. A simple 1:2:1 ratio was suggested as the logical theory to explain the data. The goodness of fit indicates that the theory was probably correct, since  $P=0.5995$ . The 1-factor difference is the more common result with spike density, but a few cases have been reported where a more complicated type of inheritance was observed (7, 9, 11, 12, 13).

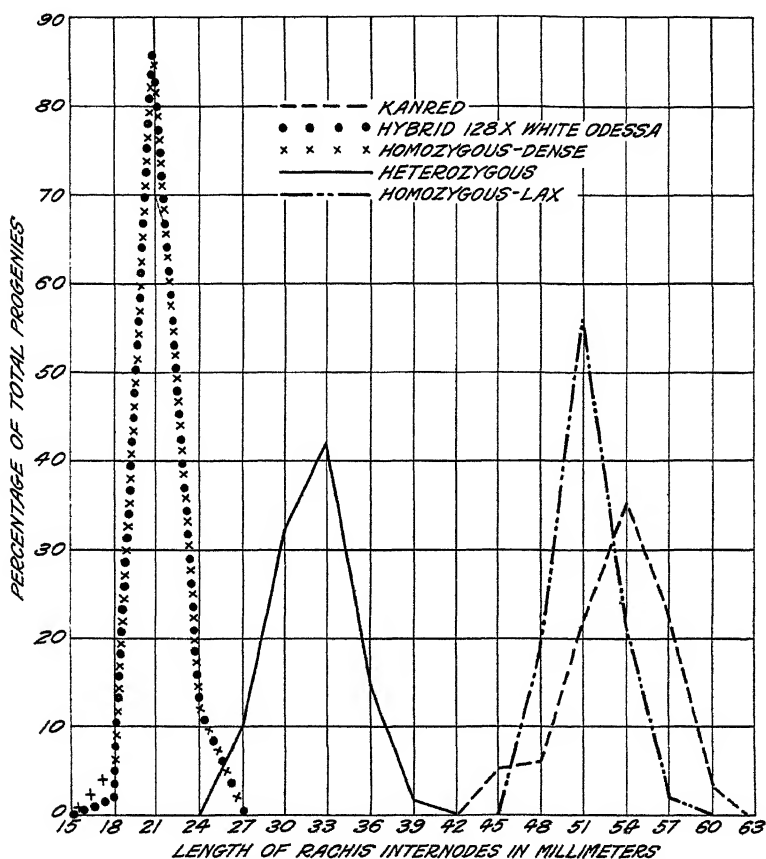


FIGURE 3.—Spike-density curves of Kanred and Hybrid 128  $\times$  White Odessa parents, and of the three progeny groups—homozygous-dense, heterozygous, and homozygous-lax

In Table 5 the goodness of fit is presented as compared to a 1:2:1 ratio.

TABLE 5.—Goodness of fit of three groups of  $F_3$  progenies for spike density compared with a 1:2:1 ratio

Progeny group	Calculated value (C)	Observed value (O)	O-C	(O-C) <sup>2</sup>	$\frac{(O-C)^2}{C}$
Homozygous-dense	53.5	65	6.5	42.25	0.7222
Heterozygous	117	111	-6	36.00	.3077
Homozygous-lax	53.5	58	-0.5	.25	.0043

$$\chi^2 = 1.0342. \quad P = 0.5995.$$



## EFFECT OF BUNT ON SPIKE DENSITY

Smutty plants were avoided in taking the data for spike density, as it was obvious that they were not normal in this respect. Data are presented to show the actual lengthening effect of bunt on spike density, the writers not having observed any previous report to this effect.

Casual observation of an infected homozygous-dense progeny might lead one to pronounce it segregating in spike density, when in reality the presence of bunt might have caused a considerable lengthening of the infected spikes as compared with normal ones on the same plant.

The plants used in this study represent many of the progenies, and as a majority of the spikes were either all smutted or all smut-free, it was difficult to get a large number of plants that had both smutty and smut-free spikes. Often the culms bearing smutty spikes were very much shorter than normal ones and seemed stunted, but in such cases they were not measured. Only those plants that produced spikes of apparently equal vigor and of the same general height were chosen.

A total of 371 plants were measured for spike density on both normal and smutty spikes. Of these 142 were homozygous for dense spikes, 83 were heterozygous, and 146 were homozygous for lax spikes.

The increase in the length of spikelet internodes caused by the presence of smut, as shown by the mean value for the group based on the measurement of 10 rachis internodes, was found to be 74 per cent in the homozygous-dense, 53.8 per cent in the heterozygous, and 8.1 per cent in the homozygous-lax. In the homozygous-dense, the increased length due to smut ranged from 25 to 240 per cent when the spike density of smutty heads was compared with that of normal heads from the same plant. A similar comparison within the heterozygous group showed an increased range in spike density of from 18.8 to 166.7 per cent. In both the above cases there was always an increase, 18.8 being the lowest increase in length measured. When the homozygous-lax heads were considered, 26 smutty heads were actually less dense than the normal heads of the same plant, 8 plants were the same for both normal and smutty spikes, while 112 smutty heads were from 1 to 132 per cent longer than corresponding normal heads on the same plant.

In order to test the significance of these figures from a different angle, computations were made by student's formula. The table used was prepared primarily for small numbers of comparisons so that the plants in each group were broken up into units and put through the formula, and the results were then averaged. The unit number chosen in this case was 30, which is represented by  $N$ . When  $N$  is 30 and  $Z$  is 0.8, the odds are 9,999:1 that the results are significant. The average value of  $Z$  for each group of 30 plants was found to be 2.22 for the homozygous-dense and 2.50 for the heterozygous, indicating in both cases very much greater than 10,000:1 odds that smut has a definite lengthening effect on the shorter head types. The value for  $Z$  in the case of the homozygous-lax group was 0.2, which shows odds of 5.9 : 1, indicating that the differences are not significant in this group. The effect of smut on the length of spike would not be apparent from general observation in the case of lax spike types. The effect of smut on spike density in the three spike-density classes is shown in Table 6.

TABLE 6.—Effect of smut on spike density

Head type	Normal mean head length	Mean length of smutty heads	Increase in head length	Odds
	<i>Mm.</i>	<i>Mm.</i>	<i>Per cent</i>	
Homozygous-dense.....	20.4	35.5	74.0	More than 10,000 : 1.
Heterozygous.....	27.5	42.3	53.8	Do.
Homozygous-lax.....	48.4	52.3	8.1	5.9 : 1.

## KERNEL COLOR

In the  $F_1$  progeny all the kernels were red. Of the 234  $F_2$  progenies three plants produced white kernels; the remainder produced red. No distinction in the degree of red could be detected. In the  $F_3$ , the three plants that contained the white kernels bred true, while the 231 remaining plants showed the following segregation types: 138 plants bred true for red kernels; 23 segregated 63 red : 1 white; 45 segregated 15 red : 1 white; and 25 segregated 3 red : 1 white. A 3-factor difference was suggested as best suited to explain these results.

The probable genotypic constitutions of the parents are Kanred  $AABBCC$ , and Hybrid 128  $\times$  White Odessa  $aabbcc$ , each of the dominant factors either alone or in combination producing red kernel color. The  $F_2$  genotypes obtained and  $F_3$  breeding behavior for each 64 genotypes would be as follows:

Parents  $AABBCC \times aabbcc$ .  
 $F_2$   $AaBbCc$  Segregate in  $F_3$  63 red : 1 white.

 $F_3$  breeding behavior

$F_2$	1	$AABBCC$	True for red kernels.
	2	$AABBcc$	True for red kernels.
	2	$AABbCC$	True for red kernels.
	2	$AaBBCC$	True for red kernels.
	4	$AABbCc$	True for red kernels.
	4	$AaBBCC$	True for red kernels.
	4	$AaBbCC$	True for red kernels.
	8	$AaBbCc$	Segregate 63 red : 1 white.
<hr/>			
	27		
	1	$AABbCc$	True for red kernels.
	2	$AABbcc$	True for red kernels.
	2	$AaBBcc$	True for red kernels.
	4	$AaBbcc$	Segregate 15 : 1 white.
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	9		
	1	$AAbbCC$	True for red kernels.
	2	$AAbbCc$	True for red kernels.
	2	$AabbCC$	True for red kernels.
	4	$AabbCc$	Segregate 15 red : 1 white.
<hr/>			
	9		
	1	$aaBBCC$	True for red kernels.
	2	$aaBBcc$	True for red kernels.
	2	$aaBbCC$	True for red kernels.
	4	$aaBbCc$	Segregate 15 red : 1 white.
<hr/>			
	9		

1	$AAbbcc$	True for red kernels.
2	$Aabbcc$	Segregate 3 red : 1 white.
<hr/>		
3		
<hr/>		
1	$aaBBcc$	True for red kernels.
2	$aabBcc$	Segregate 3 red : 1 white.
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3		
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1	$aabbCC$	True for red kernels.
2	$aabbCc$	Segregate 3 red : 1 white.
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3		
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1	$aabbcc$	True for white kernels.
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64		

On a basis of three factors for red kernel color, for each 64 plants the following groups and numbers should be obtained:

True-breeding red grain.....	37
Segregating 63 red : 1 white.....	8
Segregating 15 red : 1 white.....	12
Segregating 3 red : 1 white.....	6
True-breeding white grain.....	1
<hr/>	
Total.....	64

When this theoretical expectation was compared with the actual data, a very close fit was obtained. The group segregating 63 red : 1 white showed widest variance from the expected number, due mainly to the fact that few of the rows furnished over 40 plants, and in some cases more than half the plants of a progeny were completely smutty, thus making the chances unfavorable for the recovery of the 1 white-kernelled plant. Stewart (12) found that many of the errors due to lack of sufficient plants in  $F_3$  can be overcome by growing an  $F_4$  generation to uncover doubtful  $F_3$  segregation.

There was little doubt as to the breeding behavior of the 15 red : 1 white and of the 3 red : 1 white groups, as from 7 to 13 plants with white kernels would be found in the 3 red : 1 white group and from 2 to 4 plants with white kernels in the 15 red : 1 white group out of a total of 40 plants used as a basis for classifying each progeny row.

The goodness of fit of the  $F_3$  progenies on the basis of a 3-factor difference are presented in Table 7.  $\chi^2 = 1.8485$  and  $P = 0.7607$ , which is a highly satisfactory fit.

TABLE 7.—*Closeness of fit of five groups of  $F_3$  progenies based on a 3-factor difference in the parents, with a 37:8:12:6:1 ratio for grain color*

Group	Calculated value (C)	Observed value (O)	(O-C)	(O-C) <sup>2</sup>	$\frac{(O-C)^2}{C}$
Homozygous red grain.....	135.27	138	2.73	7.45	0.0551
Segregating 63 red : 1 white.....	29	23	-6	36	1.2414
Segregating 15 red : 1 white.....	44	45	1	1	.0227
Segregating 3 red : 1 white.....	22	25	3	9	.4091
Homozygous white grain.....	3.66	3	-.66	.44	.1202

$\chi^2 = 1.8485$ .  $P = 0.7607$ .

An attempt was made by inspection to classify the grain according to the number of factors for red. No evidence was found that kernels with two or three factors for red differed in intensity of color from those having only one factor.

#### SUMMARY

Kanred, one of the best winter wheats in the intermountain region, was crossed with Hybrid 128×White Odessa, an introduction from the Washington experiment station. From a group of  $F_1$  plants the most vigorous one was chosen to continue the experiment. The  $F_1$  generation from this cross was intermediate for awns and for spike density, but the kernel color was red, resembling closely that of the Kanred parent. An  $F_2$  family containing 234 plants was obtained, and the grain from each plant was used to seed one  $F_3$  progeny row. The kernels were spaced approximately 3 inches apart in the row and the rows were 12 inches apart. Each row was about 12 feet long, and contained from 40 to 60 plants.

As the primary purpose in making the cross was to develop a variety of wheat resistant to bunt (*Tilletia tritici*), only one row of the Hybrid 128×White Odessa, the resistant parent, and one row of Kanred were included.

The 234  $F_3$  progenies were studied for the various character differences with the idea of obtaining the genetic constitution of the  $F_2$  plants more accurately than could be determined by studying the  $F_2$  plants themselves.

Data were collected for the following plant characters, either by observation or measurement: (1) Awn classes; (2) spike density, including the effect of bunt on the density of spike; and (3) color of kernel.

Awn classes were found to be inherited on the basis of a single-factor difference. Both parental types were recovered in the homozygous progenies of the  $F_3$  generation. When the three genotypic  $F_3$  progeny groups were compared to a 1:2:1 ratio, a very good fit was obtained,  $P=0.8190$ .

Spike density has been found to be rather stable even in years of fluctuating environment, and in this data 10 check rows of Kanred grown and measured a previous year were used for comparison.

Spike density was also found to be inherited on a single-factor basis. A very close fit of the expected 1:2:1 ratio to the data was obtained, with  $P=0.5995$ . Both parental types were recovered in the true-breeding  $F_3$  progenies for the character of spike density.

The effect of bunt on spike density was indicated by an increase of from 53.8 per cent in the heterozygous progenies to 74 per cent in the homozygous-dense progenies for the length of 10 internodes. When compared to Student's formula, the results of these two spike-density types were significant by infinite odds. An increase of only 8.1 per cent was found in the case of the homozygous-lax type, a difference which was not noticeable to the eye and when measured gave odds of 5.9 : 1, which is not considered significant.

Kernel-color inheritance was found to be more complex in this cross than were the other characters studied. The data taken suggested a 3-factor difference as the explanation of the behavior of kernel color. The goodness of fit was calculated for the five groups of  $F_3$  progenies,

based on a 3-factor difference, and it was found that  $P=0.7607$ , which is a good fit.

It was impossible to determine any cumulative effect of the three factors for red kernel color. The seasonal and environmental effects caused red kernels to show as much variation in a progeny as the difference between progenies differing in the number of factors present for red color.

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## METAXENIA IN COTTON<sup>1</sup>

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### INTRODUCTION

It has been observed at the United States Field Station, Sacaton, Ariz., that well-grown plants of inbred families of Egyptian cotton show occasional variations of considerable magnitude in the length of the lint borne on individual seeds. In the same boll one or more seeds may be found on which the lint is as much as one-eighth inch shorter than the average. The discovery by Swingle (9)<sup>2</sup> and Nixon (7, 8) of "metaxenia," or immediate effect of pollen on tissues of the mother plant, in the date palm suggested the possibility that the variations observed in cotton may in some cases result from cross-pollination. Search of the literature having failed to reveal any mention of what might be considered metaxenia effects in the cotton plant, it was decided to test the matter experimentally.

### EXPERIMENT IN 1928

#### MATERIAL AND METHODS

Two very distinct types of cotton, Egyptian (*Gossypium barbadense* L. ?) and Hopi (*G. hopi* Lewton), which differ greatly in the length of time required for maturation of the boll, in length of lint, and in fuzziness of seeds, were selected for the experiment. The Egyptian type was represented by a family of the Pima variety which had been strictly inbred four generations and was very uniform in all of its characters. The Hopi type, a peculiar cotton formerly grown by certain Indian tribes in Arizona, was represented by the variety designated "Sacaton," a form once cultivated by the Pima Indians along the Gila River, which differs slightly from the typical form of the species grown by the Hopi Indians in northeastern Arizona. In this case also a uniform family, inbred at least four generations, was available for the experiment.

Flowers on four of the Pima plants were marked by tags showing the date of anthesis and were left to natural pollination by their own pollen and that of surrounding plants of the Egyptian type,<sup>3</sup> while on three other Pima plants the flowers were emasculated to prevent

<sup>1</sup> Received for publication Nov. 25, 1930; issued May, 1931.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 543.

<sup>3</sup> Previous investigation had shown that even when the conditions were optimum for determining the extent of cross-fertilization, i. e., when individual plants were grown isolated in a plot of another kind of cotton, only 12 per cent of the ovules on the Egyptian plants proved to have been cross-fertilized (2, p. 2, Table 5). In the experiment here described, the Pima plants were in the center of a border containing only plants of the Egyptian type, so that such cross-pollination as may have occurred could not have affected the results appreciably.

self-fertilization and were pollinated with pollen from plants of the Hopi family. These flowers also were marked with tags showing the date of anthesis. The plants that received Egyptian pollen were less favorably situated and somewhat smaller than those that received Hopi pollen.

Tagging of the flowers was begun on July 24 and ended September 5. Only the bolls that opened before the first frost were harvested, since frosted bolls open prematurely and hence are unsuitable for determination of the maturation period.

Table 1 gives the statistical constants for the characters determined on the material from the pollinations Pima×Pima and Pima×Hopi. These were: Boll period, lint length, lint index, seed fuzziness, number of seeds per boll, and weight of seeds. The boll period was recorded as the number of days from anthesis to opening of the boll. Lint length and seed fuzziness were indicated by grades, the latter having been determined after the samples were ginned. The lint index and the weight of the seeds were determined in grams, the latter after removal of the lint. In computing these values the weight of all the seeds and of all the lint in each boll was divided by the number of seeds therein, and the quotients were multiplied by 100 in order to avoid the use of small fractions of a gram.

TABLE 1.—Statistical constants for characters determined on bolls from Pima cotton flowers naturally pollinated (Pima×Pima) and artificially cross-fertilized with Hopi pollen (Pima×Hopi)

Character and population	Determinations	Mean	Standard deviation	Coefficient of variation
<b>Boll period:</b>	<b>Number</b>	<b>Days</b>	<b>Days</b>	
Pima×Pima.....	248	62.2±0.33	7.80	12.5±0.39
Pima×Hopi.....	146	61.1±.41	7.38	12.1±.48
Difference.....		1.1±.53		.4±.62
<b>Lint length:</b>	<b>Grade</b>	<b>Grade</b>		
Pima×Pima.....	102	8.0±0.02	0.35	4.4±0.21
Pima×Hopi.....	93	7.4±.03	.40	5.4±.27
Difference.....		.6±.036		1.0±.34
<b>Lint index:</b>	<b>Grams</b>	<b>Grams</b>		
Pima×Pima.....	65	5.31±0.037	0.44	8.3±0.49
Pima×Hopi.....	57	5.38±.038	.42	7.8±.49
Difference.....		.07±.053		.5±.69
<b>Seed fuzziness:</b>	<b>Grade</b>	<b>Grade</b>		
Pima×Pima.....	102	6.6±0.08	1.20	18.2±0.89
Pima×Hopi.....	93	5.8±.10	1.37	23.6±1.23
Difference.....		.8±.13		5.4±1.52
<b>Seeds per boll:</b>	<b>Number</b>	<b>Number</b>		
Pima×Pima.....	102	16.6±0.21	3.09	18.6±0.91
Pima×Hopi.....	93	16.5±.26	3.64	22.0±1.14
Difference.....		.1±.33		3.4±1.46
<b>Weight 100 seeds:</b>	<b>Grams</b>	<b>Grams</b>		
Pima×Pima.....	101	12.7±0.06	0.89	7.0±0.33
Pima×Hopi.....	93	13.2±.06	.84	6.4±.31
Difference.....		.5±.085		.6±.45

It had been discovered in other populations of cotton plants that some of these characters are correlated, and this fact should be taken

into account in considering possible metaxenia effects. The correlations were therefore computed for all pairs of the characters determined in the two populations, with the exception of boll period, which was not recorded in such a way as to make it possible to identify the individual bolls on which the other characters were determined. The coefficients of correlation are given in Table 2.

TABLE 2.—Correlations of characters determined in populations from the fertilizations *Pima*×*Pima* and *Pima*×*Hopi* in 1928 <sup>a</sup>

Character and population	Correlation with—				
	Lint length	Lint index	Seed fuzziness	Number of seeds	Weight of seeds
Lint length:	<i>r</i>	<i>r</i>	<i>r</i>	<i>r</i>	<i>r</i>
<i>Pima</i> × <i>Pima</i> .....	-----	-0.027±0.083	+0.034±0.067	-0.057±0.067	+0.089±0.067
<i>Pima</i> × <i>Hopi</i> .....	-----	-.210±.085	+.287±.064	-.084±.069	+.190±.067
Lint index:					
<i>Pima</i> × <i>Pima</i> .....	-----	-----	-.297±.076	+.056±.083	+.217±.080
<i>Pima</i> × <i>Hopi</i> .....	-----	-----	-.123±.083	+.262±.083	-.028±.089
Seed fuzziness:					
<i>Pima</i> × <i>Pima</i> .....	-----	-----	-----	-.220±.064	+.360±.058
<i>Pima</i> × <i>Hopi</i> .....	-----	-----	-----	+.158±.068	+.074±.069
Number of seeds:					
<i>Pima</i> × <i>Pima</i> .....	-----	-----	-----	-----	-.350±.059
<i>Pima</i> × <i>Hopi</i> .....	-----	-----	-----	-----	-.193±.067
Weight of seeds:					
<i>Pima</i> × <i>Pima</i> .....	-----	-----	-----	-----	-----
<i>Pima</i> × <i>Hopi</i> .....	-----	-----	-----	-----	-----

<sup>a</sup> The number of determinations is 101 for the population *Pima*×*Pima* and 93 for the population *Pima*×*Hopi*, except in the case of lint index, for which the numbers are 65 and 57, respectively.

#### INFLUENCE OF POLLEN ON BOLL PERIOD

Maturity of the boll being a somewhat indefinite phenomenon, an arbitrary standard based on the degree of separation of the carpels was adopted and was recorded photographically. Figure 1 illustrates the standard of maturity adopted. In the fall each boll from a tagged flower was gathered as soon as it reached this stage, and the date was marked on the tag that bore the date of anthesis. The boll period, or number of days from anthesis to maturity, is the difference between the two dates. (Fig. 1.)

The parental types differ greatly in the length of the boll period. Determinations in 1928 on bolls from naturally pollinated flowers gave a mean of  $62.2 \pm 0.33$  days for *Pima* (248 bolls) and a mean of  $44.4 \pm 0.37$  days for *Hopi* (130 bolls). The mean difference, 18 days, is 36 times its probable error. The statistical constants of the period of development of bolls from *Pima* flowers pollinated, respectively, with *Pima* and with *Hopi* pollen are given in Table 1.

The average period for bolls from *Pima* flowers cross-fertilized with *Hopi* pollen (*Pima*×*Hopi*) was one day shorter than for bolls from the mostly self-fertilized flowers of the same variety (*Pima*×*Pima*), but the difference is not significant, being only twice its probable error. A significant difference might have been observed if the bolls of the two populations had been exposed to the same conditions. Since the *Pima* plants on which the flowers were fertilized with *Hopi* pollen were more luxuriant and larger leaved, the resulting bolls were more shaded than the bolls from naturally pollinated, hence mostly self-fertilized, *Pima* flowers. It is a matter of every-day observation that bolls exposed to full sunlight open sooner than shaded bolls, so



it may be assumed that in this experiment the full effect of Hopi pollen in shortening the maturation period of the Pima bolls was not realized because of environmental factors working in the opposite direction.

#### INFLUENCE OF POLLEN ON LINT LENGTH

The length of the lint was determined on one seed from each of 195 bolls borne on Pima plants, of which 102 bolls were from naturally pollinated flowers and 93 bolls were from flowers cross-fertilized with Hopi pollen. No determinations were made in the bolls from naturally pollinated flowers on the Hopi plants, but the lint of this inbred family is rather uniformly about 22 mm. (seven-eighths of an

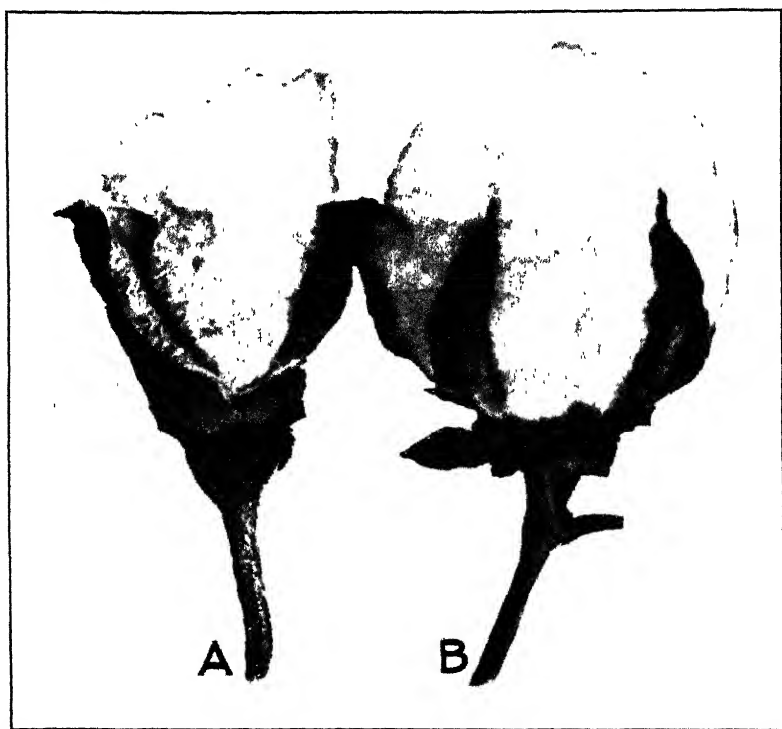


FIGURE 1.—Standard of maturity of bolls as used in this experiment: A, Pima Egyptian cotton; B, Durango upland cotton

inch) long, as compared with a mean length of 41 mm. ( $1\frac{1}{2}$  inches) in Pima. (Fig. 2.) The pollination Pima  $\times$  Pima was represented by bolls from three flowers of each of the 34 days on which flowers were tagged. In order to eliminate the influence of seasonal variations, the endeavor was made to have the 34 days equally represented also in the series of bolls from the pollination Pima  $\times$  Hopi, but there were a few days toward the end of the period when fewer than three flowers were produced by the plants that received this pollination. Every day of the 34-day period is represented in both lots, however. There were only 9 more bolls in the lot representing the Pima  $\times$  Pima pollination than in the lot representing the Pima  $\times$  Hopi pollination, and the difference in number on a few days could not have affected

the result seriously. The lint length was graded by the method described by Kearney (3, p. 10, fig. 2). Since on this scale one grade corresponds to 3.2 mm. (one-eighth of an inch), the length in millimeters or inches represented by the mean of the determinations of grade in a population is easily computed.

Comparison of the means of lint length in Table 1 shows a reduction in length of the lint on the Pima plants resulting from fertilization with pollen of Hopi, a short-linted cotton, amounting to 0.6 grade, equivalent to 1.9 mm., or slightly more than one-sixteenth of an inch. The difference is highly significant, being 16 times its probable error.

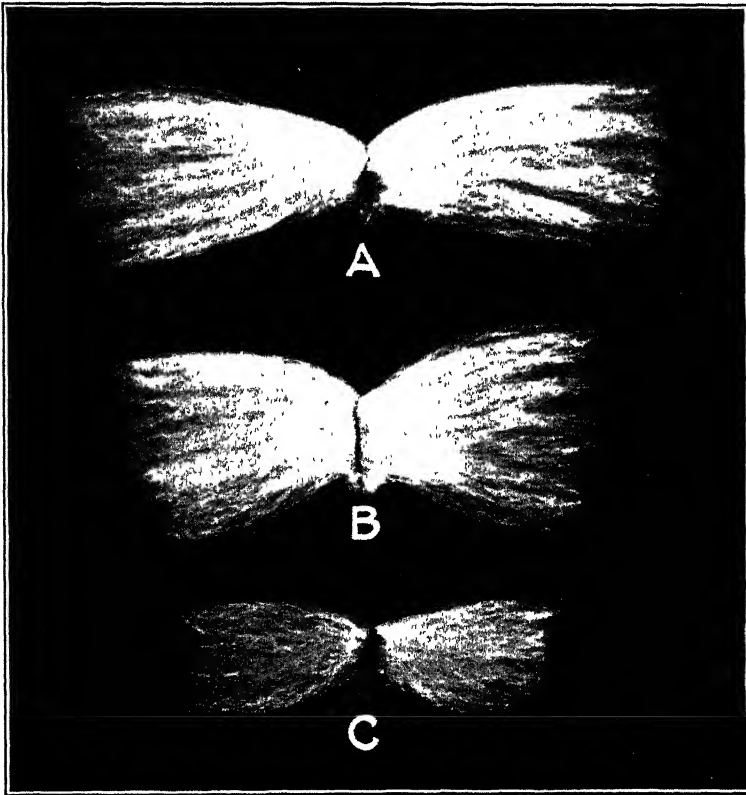


FIGURE 2.—Lint length of types of cotton used in this investigation: A, Pima Egyptian; B, Durango upland; C, Hopi. Natural size

The lint length in the bolls from the Hopi fertilization (Pima  $\times$  Hopi) was less uniform than in the bolls from naturally fertilized flowers (Pima  $\times$  Pima), but the difference between the coefficients of variation is barely 3 times its probable error.

Reference to Table 2 shows that lint length was significantly correlated with none of the other characters in the population Pima  $\times$  Pima, but gave a significant positive correlation with seed fuzziness and possibly with seed weight in the population Pima  $\times$  Hopi. Neither correlation would seem to affect the significance of the reduction in lint length by the Hopi pollen, which, as shown in Table 1, reduced

both the length of the lint and the amount of fuzz on the seeds while increasing the weight of the seeds. The absence of significant correlation between lint length and the other characters in the Pima  $\times$  Pima population permits the inference that there is no general association of these characters which would necessitate regarding the effect of the kind of pollen on the length of the lint as an indirect one.

For the reason already mentioned, the full effect of the Hopi pollen in shortening the lint on the Pima plants probably was not realized in this experiment, the individual plants whose flowers received Hopi pollen having been grown under soil conditions conducive to greater length of the lint than were the individual plants whose flowers were fertilized by pollen of the same type (Egyptian).

#### INFLUENCE OF POLLEN ON LINT INDEX

Reference to Table 1 shows that the lint index was not significantly affected by the kind of pollen used, although the parental types, Pima and Hopi, differ considerably in abundance of the lint. (Fig. 2).

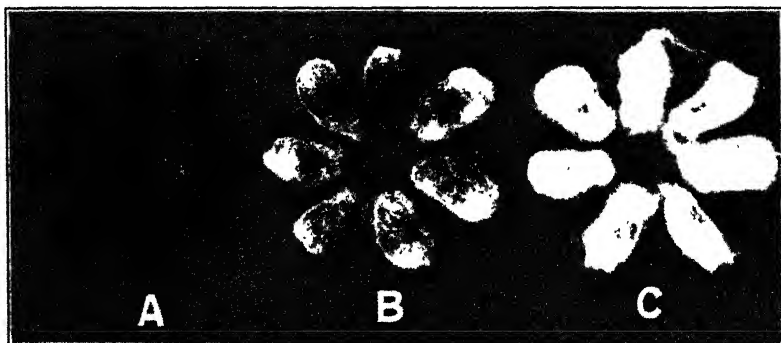


FIGURE 3.—Seeds after removal of lint: A, Hopi cotton; B, Pima cotton; C, Durango cotton. Natural size

#### INFLUENCE OF POLLEN SEED ON FUZZINESS

The family of Pima Egyptian cotton used in this experiment has decidedly fuzzy seeds, many of them having from one-half to nearly all of their surface covered with short hairs or fuzz, while the seeds of the Hopi family, after removal of the lint, are almost naked. (Fig. 3.) In view of this contrast, it was of interest to determine whether this character also is affected by the pollen. The degree of fuzziness was determined on the seeds from each of the bolls on which lint length was determined, the seeds having been compared with a series of nine samples representing the entire range of seed fuzziness observed in Egyptian cottons. The standard for grade 1 has the seeds naked except for a tuft of fuzz at the base and often a trace along the raphe, while in the standard for grade 9 most of the seeds are entirely or almost entirely covered with fuzz.

It is evident from the data in Table 1 that fertilization with pollen of Hopi, a smooth-seeded cotton, reduced the average quantity of fuzz on the seeds borne on the Pima plants by nearly a full grade. The difference is significant, being six times its probable error. As was also the case in lint length, fertilization with Hopi pollen increased the amount of variation in this character. The coefficient of variation

is significantly lower in the population Pima×Pima than in the population Pima×Hopi, the difference being 3.5 times its probable error. Reference to Table 2 shows that in the population Pima×Pima seed fuzziness was correlated significantly and negatively with lint index and with number of seeds, and significantly and positively with weight of seeds, while in the population Pima×Hopi the only significant correlation of this character was the positive one with lint length. It may be inferred from the fact that none of these pairs of characters showed significant correlation in both populations that the difference in seed fuzziness between the two populations was a direct effect of the kind of pollen and is not to be interpreted through the association of this with other characters.

#### NUMBER AND WEIGHT OF SEEDS

The number of seeds in the mature boll is determined by the initial number of ovules and by the degree of fertilization attained. No metaxenia effect is to be sought here, but the number of seeds should be considered in its relation to characters in regard to which metaxenia is indicated. Reference to Table 1 shows that the bolls of the two populations did not differ significantly in their mean number of seeds, so this could not have been a factor in the observed differences in other characters between the two populations.

Weight of the individual seeds, here expressed for convenience as mean weight per 100 seeds, is obviously not a satisfactory character for the detection of metaxenia in cotton, since in this plant almost the entire volume of the mature seed is occupied by the embryo, which is the young daughter plant rather than a part of the mother plant. The data in Table 1 show that the mean seed weight was significantly higher in the population Pima×Hopi than in the population Pima×Pima, the difference being nearly six times its probable error. It is probable that a heterosis or hybrid-vigor effect is involved, previous investigations having shown that the seeds from cross-fertilization of upland and Egyptian cottons are heavier than the seeds from fertilization within either type, and that the resulting  $F_1$  plants show pronounced hybrid vigor, even in the early seedling stages.<sup>4</sup>

Of the two characters, lint length and seed fuzziness, which are believed to have shown metaxenia in this experiment, it is seen in Table 2 that the former was not significantly correlated with weight of seeds in the population Pima×Pima, while there was a positive but scarcely significant correlation in the population Pima×Hopi. Seed fuzziness was positively and significantly correlated with weight of seeds in Pima×Pima but not in Pima×Hopi. The absence of significant correlations of the same sign in both populations makes

<sup>4</sup> In an experiment performed at the Sacaton station in 1922, the results of which have not been published hitherto, flowers of Pima Egyptian cotton were emasculated and were pollinated, some with Pima pollen and some with pollen of the Lone Star variety of upland cotton. Reciprocal pollinations were made on emasculated flowers of Lone Star upland. Determinations of the weight of the individual seeds from the resulting bolls gave the following means:

	Gram
Pima×Pima.....	0.129±0.0006
Lone Star×Lone Star.....	.126±.0006
Pima×Lone Star.....	.135±.0010
Lone Star×Pima.....	.135±.0040

The seeds from the reciprocal cross-fertilizations Pima×Lone Star and Lone Star×Pima had exactly the same mean weight, which was considerably greater than the weights of the seeds from the two fertilizations with like pollen.

it unlikely that the presumable metaxenia effect on lint length and seed fuzziness is associated with the difference in seed weight between the two populations, especially as the population which had the heavier seeds, Pima  $\times$  Hopi, had the shorter lint and the smaller development of fuzz. (See Table 1.)

#### EXPERIMENT IN 1929

It was recognized that the experiment performed in 1928 was not as conclusive as could be desired, the flowers on the control plants having been left to natural pollination instead of having been strictly self-fertilized. There is good reason to believe that, since most of the ovules in naturally pollinated flowers are self-fertilized, and since the Pima plants in this experiment were surrounded by plants of this and other long-linted varieties of Egyptian cotton, such cross-pollination as may have occurred could not have vitiated the results appreciably. Nevertheless, before concluding that so remarkable a phenomenon as metaxenia occurs in cotton, it was desirable to obtain evidence from a more carefully controlled experiment.

#### MATERIAL AND METHODS

Three varieties, each belonging to a different species, were chosen for this experiment. These were the Pima variety of Egyptian cotton (*Gossypium barbadense* L. ?), the Durango variety of upland cotton (*G. hirsutum* L.), and Hopi cotton (*G. hopi* Lewton), the last being represented by the typical form grown by the Hopi Indians in north-eastern Arizona. These varieties differ markedly in the time required for maturation of the boll and in length of the lint. The mean maturation period of Pima, as determined in 1928, is 62.2 days; of Durango as determined by King and his colleagues (6) in 1924, 47.4 days; and of Hopi, as determined in 1928, 44.4 days. The length of the lint averages about 41 mm. (1½ inches) in Pima, 32 mm. (1¼ inches) in Durango, and 22 mm. (seven-eighths of an inch) in Hopi. Inbred and uniform families of the Pima and Hopi varieties and plants of the Durango variety grown from the purest commercial seed obtainable were used for the experiment. All pollinations were made on Durango plants, this variety being approximately intermediate in lint length and having a boll-maturation period somewhat longer than that of Hopi and much shorter than that of Pima.

Durango plants to the number of 25, showing a thrifty and uniform growth, were selected. All flower buds due to open the following day on these plants were emasculated each evening and bagged to exclude insects, thus preventing unguarded cross-pollination. Flower buds which were to furnish pollen were bagged at the same time on the Pima, Durango, and Hopi plants, this precaution having been taken in order to prevent any admixture of foreign grains in the pollen used. The pollinations were made the following morning between the hours of 10 a. m. and noon, care being taken to apply enough pollen to insure the maximum attainable degree of fertilization.<sup>5</sup>

It was important to eliminate the influence of seasonal and positional variations, to which the characters of the cotton plant are peculiarly subject. It is well known, for example, that the time required for maturation of the boll is many days shorter for flowers produced early in the season than for the late summer flowers (6, p. 948). Position on the plant is also a factor influencing maturity

<sup>5</sup> The writer is indebted to H. J. Fulton and Warren Hastings for valuable assistance in these operations.

to an important extent. A boll situated so as to receive full sunlight will sometimes mature in 10 days' less time than a boll, originating on the same date, that has been shaded at all times. Lint and seed characters also are affected by position, Kearney and Harrison (5) having found that in Pima cotton the lint is shorter and the seeds are fuzzier in bolls borne near the base of the plant than in bolls borne higher. Here there is obviously a correlation between positional and seasonal effect, the "bottom" bolls being produced by very early flowers and the upper bolls by later flowers.

To avoid the influence of changes in conditions with the advance of the season, the ideal plan would have been to give each of the three pollinations to an equal number of flowers on each of the Durango plants on each day of the experiment. Since none of the plants produced as many as three flowers every day, this ideal was unattainable. The plan adopted was to keep an accurate record of the pollinations performed daily and to follow a definite rotation. An example will show how the plan operated. Assuming that Durango plant No. 1 produced two flowers on the first day of the experiment, one flower on the second day, and three flowers on the third day and that on the first day one flower was pollinated with Pima and one with Hopi pollen, then the one flower produced on the second day would be pollinated with Durango pollen, and one each of the three flowers produced on the third day would receive Pima, Hopi, and Durango pollen, respectively. It is believed that this procedure, which was followed systematically throughout the experiment, equalized the influence of both seasonal and positional variations and that theseveral lots of bolls representing the different pollinations were strictly comparable.

The first pollinations were made on July 19 and the last from which bolls matured before frost were made on September 5. The number of days on which pollinations were made during this period was 49. Owing to the lateness of the spring, it so happened that this period represented practically all of the active fruiting life of the Durango variety in 1929. Bolls that had not reached the accepted standard of maturity before frost occurred were disregarded.

All of the characters determined in the earlier experiment were measured in the experiment of 1929 with the exception of seed fuzziness. Owing to the fact that the female parent, Durango, has entirely fuzzy seeds (fig. 3), it was found impracticable to measure such variations as may have occurred in this character. The statistical constants of the five characters are given in Table 3, and the coefficients of the correlations among them in Table 4.

The populations on which the statistical constants were determined comprised bolls from 49 days of flowering in this experiment and from 44 days of flowering in the experiment of 1928. These periods represent a considerable range of seasonal conditions, and evidence has been obtained in other investigations that the length of the boll period, length of the lint, and degree of fuzziness of the seeds vary appreciably according to the time of flowering (4, 5, 6). These seasonal variations must have had the effect of increasing the standard deviations and probable errors. There is little doubt that the significance of the observed differences would have been greater if the bolls representing the several fertilizations had been produced during a shorter period of flowering.

TABLE 3.—Statistical constants for characters determined on bolls from emasculated flowers of Durango upland cotton fertilized with pollen of Pima Egyptian, Durango upland, and Hopi cotton, respectively

Character and population	Determinations	Mean	Standard deviation	Coefficient of variation
<b>Boll period:</b>	<i>Number</i>	<i>Days</i>	<i>Days</i>	
Durango×Pima.....	123	54 0±0 19	3.18	5.9±0.25
Durango×Durango.....	117	52 9±.20	3.16	6.0±.26
Durango×Hopi.....	124	52 5±.22	3.64	6.9±.30
<b>Lint length:</b>		<i>Grade</i>	<i>Grade</i>	
Durango×Pima.....	100	5.3±.05	.73	13.8±.67
Durango×Durango.....	100	4.9±.04	.64	13.2±.64
Durango×Hopi.....	100	4.6±.06	.90	19.4±.97
<b>Lint index</b>		<i>Grams</i>	<i>Grams</i>	
Durango×Pima.....	100	6.49±.064	.95	14.6±.71
Durango×Durango.....	100	6.29±.051	.75	11.9±.58
Durango×Hopi.....	100	6.23±.062	.92	14.8±.72
<b>Seeds per boll:</b>		<i>Number</i>	<i>Number</i>	
Durango×Pima.....	100	24.4±.34	5.05	20.7±1.03
Durango×Durango.....	100	28.1±.30	4.51	16.3±.80
Durango×Hopi.....	100	28.1±.39	5.72	20.6±1.02
<b>Weight 100 seeds:</b>		<i>Grams</i>	<i>Grams</i>	
Durango×Pima.....	100	14.0±.11	1.62	11.6±.55
Durango×Durango.....	100	13.2±.08	1.18	9.0±.43
Durango×Hopi.....	100	13.0±.09	1.32	10.1±.48

TABLE 4.—Correlations of characters determined in populations from the fertilizations Durango×Pima, Durango×Durango, and Durango×Hopi in 1929<sup>a</sup>

Character and population	Correlation with—				
	Boll period	Lint length	Lint index	Number of seeds	Weight of seeds
Boll period:	<i>r</i>	<i>r</i>	<i>r</i>	<i>r</i>	<i>r</i>
Durango×Pima.....	-----	+0.113±0.067	+0.258±0.063	+0.171±0.065	+0.047±0.067
Durango×Durango.....	-----	-.060±.067	+.358±.059	+.073±.067	+.108±.067
Durango×Hopi.....	-----	-.079±.067	+.135±.066	-.009±.067	+.006±.067
Lint length:	+0.113±0.067	-----	-.055±.067	-.011±.067	+.045±.067
Durango×Pima.....	-----	-.060±.067	+.042±.067	+.128±.066	-.045±.067
Durango×Durango.....	-----	-.079±.067	+.012±.067	-.018±.067	+.229±.067
Lint index:	+.258±.063	-.055±.067	-----	-.084±.067	+.231±.064
Durango×Pima.....	+.358±.059	+.042±.067	-----	+.009±.067	+.259±.063
Durango×Durango.....	+.135±.066	+.012±.067	-----	-.126±.066	+.246±.063
Number of seeds:	+.171±.065	-.011±.067	-.084±.067	-----	-.457±.053
Durango×Pima.....	+.073±.067	-.128±.066	+.009±.067	-----	-.273±.062
Durango×Durango.....	-.009±.067	-.018±.067	-.126±.066	-----	-.174±.065
Durango×Hopi.....	-----	-----	-----	-----	-----
Weight of seeds:	+.047±.067	+.045±.067	+.231±.064	-.457±.053	-----
Durango×Pima.....	+.108±.067	-.045±.067	+.239±.063	-.273±.062	-----
Durango×Durango.....	+.006±.067	+.229±.064	+.246±.063	-.174±.065	-----
Durango×Hopi.....	-----	-----	-----	-----	-----

<sup>a</sup> The number of determinations is 100 for each character in each population.<sup>b</sup> Omitting 5 very inferior bolls containing only 11 or 12 seeds and grading only 1.0 to 2.0 in lint length, or the remaining 95 bolls is reduced to + 0.072±0.069.

## INFLUENCE OF POLLEN ON BOLL PERIOD

On the 25 Durango plants a total of 1,072 flowers were emasculated and pollinated, 359 with Pima pollen, 351 with Durango pollen, and 362 with Hopi pollen. The date of maturation of the boll was determined as in the experiment of 1928. The plants were inspected daily during the fall, and all bolls that had reached the stage of opening indicated by the standard of maturity were picked promptly.

The numbers of mature bolls obtained from the several pollinations were approximately equal, having been respectively 123, 117, and 124. The number maturing on the individual plants ranged from 7 to 25 and averaged 14.5. All bolls from a given pollination on all of the plants were taken as one array in computing the mean period of maturation.

The period from anthesis to opening of the boll on the Durango plants, as indicated by the data in Table 3, averaged 1.5 days longer from flowers fertilized with pollen of Pima Egyptian, a variety having a long maturation period, than from flowers fertilized with pollen of Hopi, a variety having a short maturation period. The difference is significant, being five times its probable error. Fertilization with Pima pollen also shows a significant retarding effect in comparison with the results of fertilization with pollen of Durango, which has a boll period somewhat longer than that of Hopi but much shorter than that of Pima. In this case the difference averages a little more than one day and is four times its probable error.

Fertilization with Hopi pollen showed a tendency to hasten the maturation of the bolls, but the mean difference between the populations Durango  $\times$  Durango and Durango  $\times$  Hopi is less than one-half day and is not significant, being little greater than its probable error. Nevertheless, it should be recalled that in the experiment of 1928 there was a slight although seemingly not significant shortening of the period of maturation of the bolls on Pima plants when the flowers were fertilized with Hopi pollen, as compared with the results of the fertilization Pima  $\times$  Pima. The consistency of the results of the two experiments gives some warrant for the assumption that the pollen of the early-maturing Hopi variety tends to hasten the maturation of the boll when used in fertilizing the flowers of more slowly maturing types of cotton.

A tendency to greater variation in the boll period of individual bolls from fertilization with Hopi pollen than from the other fertilizations is indicated by comparison of the coefficients of variation, but the differences are not significant.

Only one of the other characters here considered, lint index, was significantly correlated with the boll period. As shown in Table 4, the coefficients were positive in all three populations and were significant in two of them. The relation between these characters does not decrease the probability that different pollens affect directly the length of the boll period, the correlation implying that the more slowly maturing bolls have more abundant lint on the seeds.

#### INFLUENCE OF POLLEN ON LINT LENGTH

The lint length was determined, by the method used in the 1928 experiment, on each of 100 bolls resulting from each pollination. In selecting the bolls for these determinations it was sought to avoid variables due to individual differences among the plants and to development of the bolls at different times. The bolls of each lot, therefore, were selected so as to represent equally, as nearly as possible, the 25 plants for the entire period during which the pollinations were made. The relative length of lint of the three parent varieties is shown in Figure 2.

The data in Table 3 show that the lint on the Durango plants averaged 0.7 grade (2.2 mm.) longer in bolls from flowers fertilized



with pollen of Pima Egyptian, the longest linted of the three varieties, than in bolls from flowers fertilized with Hopi, the shortest-linted variety. The difference between the means is highly significant, being about nine times its probable error. An appreciable and significant lengthening of the lint on the Durango plants, due to fertilization with Pima pollen, is shown also by comparing the fertilizations Durango  $\times$  Pima and Durango  $\times$  Durango. The difference averages 0.4 grade (1.3 mm.) and is about six times its probable error. A smaller but significant shortening effect of the Hopi pollen is found when the fertilizations Durango  $\times$  Durango and Durango  $\times$  Hopi are compared. The difference between the means in this case is 0.3 grade (1.0 mm.) and is four times its probable error.

Since the weight of the seeds as well as the length of the lint was significantly greater in the population Durango  $\times$  Pima than in the other

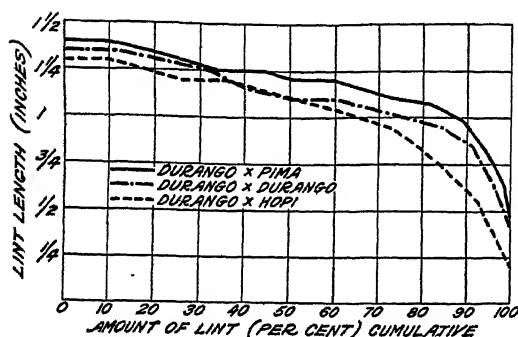


FIGURE 4.—Cumulative frequency curves showing the immediate effect of the several kinds of pollen on the length of lint on the Durango cotton plant

Comparison of the coefficients of variation in Table 3 shows no significant difference in the variation of lint length from boll to boll as between the fertilizations Durango  $\times$  Pima and Durango  $\times$  Durango, but the lint from the fertilization Durango  $\times$  Hopi was significantly more variable than that from either of the other fertilizations.

The means of lint length as given in Table 3 are based on rather rough determinations of the length of the bulk of the fibers on individual seeds, one from each boll. The method gives a result essentially the same as in commercial classing for length. It is well known, however, that in the most uniform cottons, the fibers on a seed are not of equal length, there being always, in fact, considerable variation. It was therefore desirable to ascertain the actual lengths in samples representing each of the populations. This was done with the Baer sorter, an instrument by means of which the lint in a sample can be divided into a series of subsamples according to length. The relation each subsample bears to the whole sample is determined and is expressed in percentages. It is then a simple matter to draw a cumulative curve showing the percentages of the various lengths of fiber in the sample. Such curves, based on samples representing each of the three populations, are shown in Figure 4. In interpreting these curves it should be understood that each successive figure on the abscissa from left to right indicates the percentage of the total sample which is of the length indicated, or longer. Taking as an

populations, the possibility suggests itself that the greater length of the lint hairs was merely a response to better nutrition afforded by the heavier seeds. But in the experiment of 1928 the lint was significantly shorter, although the seeds were significantly heavier, in the population Pima  $\times$  Hopi than in the population Pima  $\times$  Pima. (Table 1.)

example the curve for Durango  $\times$  Pima, it is seen that somewhat more than 40 per cent of the sample is  $1\frac{1}{4}$  inches or longer and that 70 per cent of the sample is  $1\frac{1}{2}$  inches or longer.

The samples representing the several pollinations were all taken from the same Durango plant. Each sample consisted of the lint from a single boll which had developed from a flower pollinated on August 12 or 13. The lint lengths in the samples representing the several pollinations ranged as follows:

Durango $\times$ Pima.....	0. 45-1. 40 inches (11. 4-35. 5 mm.)
Durango $\times$ Durango.....	0. 25-1. 35 inches ( 6. 3-34. 3 mm.)
Durango $\times$ Hopi.....	0. 15-1. 35 inches ( 3. 8-34. 3 mm.)

The longest fibers in the three samples differed little in length, but the shortest fibers from the fertilization Durango  $\times$  Pima were three times as long as the shortest fibers from the fertilization Durango  $\times$  Hopi. The shortest fibers from the Hopi fertilization were only three-fifths as long as the shortest from fertilization with pollen of the same variety (Durango), and were, in fact, almost short enough to be classed as fuzz hairs or linters. Since Hopi plants which supplied the pollen, did not show these very short hairs, and since they did not appear on the Durango seeds resulting from fertilization with like pollen, a metaxenia effect not foreshadowed in the expressed characters of either parent is evident in this case.

Lint length was not significantly correlated with any other character determined in the three populations, as is evident from the data given in Table 4. The apparently significant positive correlation with seed weight in the Durango  $\times$  Hopi population was due wholly to the inclusion of five stunted bolls on a single plant, which contained exceptionally few and light seeds with abnormally short lint, grading only 1.0 to 2.0 as compared with a mean grade of 4.6 in this population. The evidence, therefore, warrants the conclusion that a metaxenia effect on this character has been demonstrated.

#### INFLUENCE OF POLLEN ON LINT INDEX

The amount of lint on the seed is indicated by the lint index, or weight of lint per seed. In the present investigation, this value was determined for each of the 100 bolls from each of the three pollinations by dividing the total weight of lint in each boll by the number of seeds therein and multiplying the quotient by 100. The three parental families bear a relation with respect to lint index different from that with respect to boll period and lint length, Durango averaging highest (lint index, 6.29), Hopi lowest (lint index, 1.92), and Pima occupying an intermediate position (lint index, 5.25). The relative abundance of the lint in the three families is fairly well shown in Figure 2.

The differences among the mean lint indices of the populations resulting from the several pollinations as given in Table 3 are scarcely significant, although the mean for Durango  $\times$  Pima exceeds the mean for Durango  $\times$  Hopi by an amount that is 2.9 times the probable error of the difference. The difference between the mean indices of the populations Durango  $\times$  Pima and Durango  $\times$  Durango is 2.4 times its probable error. If the latter difference had been significant, the position would be an interesting one, indicating an increase in the abundance of lint on the Durango seeds as a result of fertilization by pollen of Pima, a variety having a somewhat lower lint index.

On the other hand, fertilization by pollen of a variety having a much lower lint index (Durango  $\times$  Hopi) did not cause an appreciable decrease in the lint index as compared with that obtained from fertilization with like pollen (Durango  $\times$  Durango). Hence, even if it could be concluded that this character was influenced by the kind of pollen, the effect did not parallel the parental relations as in the case of boll period, lint length, and seed fuzziness. The analogy is rather with the phenomenon of heterosis, or hybrid vigor, which is usually greater the wider the cross, since fertilization by pollen of Pima, a very distinct species, increased the lint index of Durango, while fertilization by pollen of Hopi, a much more nearly allied species, had no appreciable effect.

It seems unlikely, at first glance, that heterosis could manifest itself in the abundance of the lint hairs which are outgrowths of the epidermis of the seed coat and therefore belong to the body of the mother plant. Evidence was given on a preceding page, however, that heterosis in cotton is shown in increased weight of the seeds on the mother plant. It is probable that the increase is mainly in the weight of the embryo or young daughter plant; but if, as seems likely, increase in weight of the embryo is accompanied by an increase in its volume, a corresponding increase in the area of the seed coat would be a physiological necessity. If increase in the area of the outer seed coat is accompanied by an increase in the number of epidermal cells, an increase in the number of lint hairs, and consequently in the lint index, would be expected.

Reference to Table 3 shows that both the lint index and the mean weight of the seeds were highest in the population Durango  $\times$  Pima.<sup>6</sup> The coefficients of correlation in Table 4 show that these characters were positively and significantly correlated in all three populations. It therefore seems possible that the observed differences in abundance of the lint, assuming them to be significant, are an indirect manifestation of heterosis, rather than of metaxenia in the strictest sense. It is interesting that heterosis was shown in seed weight and possibly in lint index in the cross-fertilization Durango  $\times$  Pima but not in the cross-fertilization Durango  $\times$  Hopi. The former represents a cross between species belonging to very different groups, Pima Egyptian representing the South American tree cotton group and Durango upland the Mexican-Central American group. Hopi cotton also belongs to the latter group, so the cross Durango  $\times$  Hopi is between much more closely related cottons than the cross Durango  $\times$  Pima and would be expected to show heterosis in a smaller degree, if at all.<sup>7</sup>

The negative correlations between weight and number of the seeds, highest in the population Durango  $\times$  Pima, as shown in Table 4, should be considered also in this connection. The mean number of seeds per boll was significantly lower in the Durango  $\times$  Pima population than in the others (Table 3), so that the greater weight of the Durango  $\times$  Pima seeds, in part at least, may have been due to better nutrition because of their smaller number. It is impossible to decide the relative importance of the two factors, heterosis and nutrition, in bringing

<sup>6</sup> In mean weight of seeds the differences were highly significant, Durango  $\times$  Pima having exceeded Durango  $\times$  Durango and Durango  $\times$  Hopi by amounts equal, respectively, to six and seven times the probable error of the difference.

<sup>7</sup> Populations representing  $F_1$  and  $F_2$  of the crosses Pima  $\times$  Hopi and Ancla upland  $\times$  Hopi were compared at Sacaton in 1928 and 1929. The former cross showed much more pronounced hybrid vigor in the  $F_1$  plant and much greater diversity in the  $F_2$  than the latter cross, in which these manifestations were scarcely more pronounced than in crosses between two varieties of upland cotton.

about the greater weight and presumably greater volume of the seeds in this population, but the possibility that the observed difference in lint index is a function of the difference in weight of the seeds is not affected by this uncertainty.<sup>8</sup>

It might be thought that the same line of reasoning would apply also to seed fuzziness, since the fuzz, like the lint, consists of simple outgrowths of the cells of the outer epidermis of the seed coat. In other words, if heterosis manifests itself in an increase of the lint index, it would be expected to manifest itself in increased fuzziness of the seeds. But it was observed in the experiment of 1928 (Table 1) that the seeds on the Pima plants in bolls resulting from cross-fertilization with pollen of another species, Hopi, were both significantly heavier and significantly less fuzzy than in bolls resulting from fertilization with Pima pollen. On the other hand, no significant difference in lint index was detected in this experiment, and lint index and seed fuzziness were negatively correlated in both populations, significantly so in Pima  $\times$  Pima.<sup>9</sup> (Table 2.) A possible explanation of the absence of correlation is that, while lint abundance was determined by weight, seed fuzziness was determined by grades based not on the weight of the fuzz hairs but on the relation of the fuzz-covered portion of the seed coat to its total area.

Comparison of the coefficients of variation in Table 3 shows that there was less variation in lint index in the population from the fertilization Durango  $\times$  Durango than in either of the populations from fertilizations with unlike pollen. Each of the differences,  $C$  of Durango  $\times$  Pima minus  $C$  of Durango  $\times$  Durango and  $C$  of Durango  $\times$  Hopi minus  $C$  of Durango  $\times$  Durango, is practically three times its probable error. Here again the position is different from that observed in respect to boll period and lint length, fertilization with Hopi pollen not having resulted in greater variation in lint index than was induced by fertilization with Pima pollen.

#### EXPERIMENT IN 1930

Acala is the only variety of upland cotton grown extensively in those parts of Arizona where Pima Egyptian cotton is grown, and it is therefore of particular interest to determine whether there are immediate effects of pollen resulting from cross-fertilization between these varieties. An experiment was performed at the United States Field Station, at Sacaton, Ariz., in 1930 to determine whether metaxenia occurs when Pima flowers receive Acala pollen and vice versa.

#### MATERIAL AND METHODS

Strictly inbred families containing the required number of plants were not available for this experiment, so plants grown from "bulk" seed were used, excluding any that were not typical of the respective variety. Selection was made of 54 plants of each variety, which were numbered consecutively. All bolls that had set previously on these

<sup>8</sup> Significant negative correlations between the number and the mean weight of the seeds in individual bolls of Pima cotton at Sacaton, Ariz., were detected by Kearney in populations grown in 1925, 1927, and 1928, the coefficients having ranged from  $-0.287 \pm 0.043$  to  $-0.413 \pm 0.037$ . An obvious physiological relation is involved in this tendency for fewer seeds to be better nourished and consequently heavier than more numerous seeds.

<sup>9</sup> There seems, however, to be no general association of these characters, for Kearney, who determined the correlation in four Pima populations, each grown in a different year, obtained in only one population a coefficient that was apparently significant, having been 3.2 times its probable error. In this case also the correlation was negative.

plants were removed before the experiment was begun. This was done because it has been found that removal of the bolls produced by the earlier flowers materially reduces boll-shedding from the later flowers (1).

The pollinations were made from August 19 to August 28, inclusive. As in previous experiments, all flowers were emasculated the preceding afternoon and pollinated between 10 a. m. and noon of the morning of anthesis. In order to insure ample material, 50 flowers on the Acala plants and 30 flowers on the Pima plants were emasculated and pollinated daily. During the 10-day period of the experiment the desired quotas were obtained on all but one or two days. On the plants of each variety one-half of the flowers were pollinated with their own kind of pollen and the remainder were pollinated with pollen of the other type. The total numbers of flowers pollinated were as follows: Acala  $\times$  Acala, 243; Acala  $\times$  Pima, 243; Pima  $\times$  Pima, 147; Pima  $\times$  Acala, 147.

Since shedding was less than was anticipated, more material was available at the end of the season than could be handled readily, and it was decided to use only 100 bolls from each pollination of each variety. In order to avoid selection, the bolls, when mature, were collected as follows: Beginning with Pima plant No. 1 and with Acala plant No. 1, all bolls from each pollination were collected, and this was repeated on the successively numbered plants until the desired number of bolls had been obtained. Since on every plant the numbers of bolls from the two pollinations were approximately the same, the individual plants of each variety contributed nearly equal numbers of bolls to the lots representing the two pollinations. Any differences that may have existed among the individual plants were equalized by this method of collection.

The procedure was varied in the case of the Acala plants by collecting separately and in equal number bolls so placed as to be fully exposed to sunlight and bolls so placed as to be shaded during most of the day. This was done in order to ascertain the effect of such difference in positions on the boll period and the length of the lint. Since the plants were spaced approximately 12 inches apart in the row, they had made rather heavy vegetative growth, and as a result almost equal numbers of bolls on all of them were exposed and shaded, respectively. Fifty bolls were collected, representing each sub-population of the Acala variety, as follows: Acala  $\times$  Pima, exposed; Acala  $\times$  Pima, shaded; Acala  $\times$  Acala, exposed; Acala  $\times$  Acala, shaded.

The characters determined on the material from this experiment were boll period (number of days from anthesis to opening of the boll) and lint length in both varieties and seed fuzziness in the Pima variety. Since all seeds produced on the Acala plants were entirely covered with fuzz, it proved impracticable to determine differences in respect to the last character in that variety. The determinations were made in the same manner as in the experiments of 1928 and 1929. The statistical constants for the several populations are given in Table 5. The table is arranged so that the upper portion contrasts the effects of the two kinds of pollen in each variety and the lower section contrasts the effects of a fully exposed and of a shaded position of the boll in the Acala variety.

TABLE 5.—Statistical constants for characters determined on bolls from emasculated flowers of *Pima Egyptian* and *Acala upland* cottons, fertilized with pollen of the same and of the other variety

Character and population	Determinations	Mean	Standard deviation	Coefficient of variation
With reference to pollination:				
Boll period—	Number	Days	Days	
Pima×Pima.....	100	74.9±0.20	2.98	4.0±0.19
Pima×Acala.....	100	73.8±.22	3.31	4.5±.21
Difference.....		1.1±.30		.5±.28
Acala×Pima.....	100	69.3±.40	5.89	8.5±.40
Acala×Acala.....	100	66.6±.30	4.52	6.8±.32
Difference.....		2.7±.50		1.7±.51
Lint length—		Grade	Grade	
Pima×Pima.....	100	8.5±.02	.30	3.5±.17
Pima×Acala.....	100	7.8±.02	.35	4.5±.21
Difference.....		.7±.03		1.0±.27
Acala×Pima.....	100	5.0±.03	.40	8.0±.38
Acala×Acala.....	100	4.4±.03	.39	8.9±.42
Difference.....		.6±.04		.9±.57
Seed fuzziness—				
Pima×Pima.....	100	5.8±.08	1.12	19.3±.02
Pima×Acala.....	100	6.2±.07	.97	15.6±.74
Difference.....		.4±.11		3.7±1.18
With reference to exposure:				
Boll period—		Days	Days	
Acala×Pima, shaded.....	50	72.7±0.37	3.85	5.3±.36
Acala×Pima, exposed.....	50	66.0±.54	5.69	8.6±.58
Difference.....		6.7±.65		3.3±.68
Acala×Acala, shaded.....	50	60.2±.34	3.56	5.1±.34
Acala×Acala, exposed.....	50	63.9±.36	3.78	5.1±.34
Difference.....		5.3±.50		0±.48
Lint length—		Grade	Grade	
Acala×Pima, shaded.....	50	5.1±.04	.40	7.8±.53
Acala×Pima, exposed.....	50	5.0±.04	.39	7.8±.53
Difference.....		.1±.06		0±.75
Acala×Acala, shaded.....	50	4.5±.04	.44	9.8±.66
Acala×Acala, exposed.....	50	4.4±.03	.33	7.5±.51
Difference.....		.1±.05		2.3±.83

## INFLUENCE OF POLLEN ON BOLL PERIOD

The boll period, or number of days from anthesis to maturation, is considerably longer in *Pima Egyptian* than in *Acala upland* cotton. Data given by King, Loomis, and Varmette (6, p. 948) show that at Sacaton in 1923 the difference averaged  $7.5 \pm 1.7$  days for flowers opening during the period August 12 to 16 and  $7.8 \pm 0.9$  for flowers opening during the period August 27 to 31.<sup>10</sup> From the results of the experiments in 1928 and 1929 it would be expected that the flowers fertilized with *Pima* pollen would have a somewhat longer boll period than the flowers fertilized with *Acala* pollen. Such proved to be the case, the data in Table 5 showing that fertilization

<sup>10</sup> Data for the intervening 10 days were not obtained by these investigators, but the two periods of 5 days each mentioned above comprise the period (Aug. 19 to 28) during which the flowers used in the present experiment reached anthesis.

of Pima flowers with Acala pollen shortened the boll period by an average of 1.1 days and that fertilization of Acala flowers with Pima pollen lengthened the boll period by an average of 2.7 days. In both cases the differences, in comparison with the result from fertilization with like pollen, were significant, having been, respectively, 3.7 and 5.4 times the probable error of the difference.

Reference to the lower portion of Table 5 shows that the bolls on the Acala plants which were in shaded positions required five or six days longer to mature than the bolls which were exposed to full sunlight during most of the day. The differences between the means for exposed and shaded bolls from each pollination, Acala  $\times$  Pima and Acala  $\times$  Acala, are very significant, being in each case about 10 times the respective probable error. It seems unlikely, however, that this difference in length of the maturation period, resulting from different positions of the bolls, influenced materially the mean difference of 2.7 days caused by the kind of pollen with which the Acala flowers were fertilized, for while shading prolonged the mean boll period 6.7 days in the case of Acala flowers fertilized with Pima pollen and only 5.3 days in the case of Acala flowers fertilized with Acala pollen, the difference ( $1.4 \pm 0.8$  days) is not significant.

The pollinations in this experiment were completed within a period of 10 days, so there could have been little variation in the conditions as to length of day, temperature, etc., under which the bolls developed. Yet comparison of the coefficients of variation in Table 5 with those in Table 3 shows that while there was less variation in the boll period on the Pima plants in the 1930 experiment than in the boll period on the Durango plants in the 1929 experiment, the latter showed less variation than the boll period on the Acala plants in the 1930 experiment.

#### INFLUENCE OF POLLEN ON LINT LENGTH

There is a great difference between these cottons in average length of the lint, that of Pima averaging about  $1\frac{1}{2}$  inches (41.3 mm.) and that of Acala averaging about  $1\frac{1}{8}$  inches (28.6 mm.). From the results of the experiments of 1928 and 1929, it would therefore be expected that fertilization of Pima flowers with Acala pollen would tend to shorten the lint and that the converse effect would result from the reciprocal fertilization (Acala  $\times$  Pima). This expectation was fully realized, as is shown by the data on lint length in Table 5. In comparison with the results from fertilization with like pollen (Pima  $\times$  Pima and Acala  $\times$  Acala), the lint in the Pima bolls from fertilization with Acala pollen averaged shorter by 0.7 grade (approximately 2.2 mm., or three thirty-seconds of an inch), and the lint in the Acala bolls from fertilization with Pima pollen averaged longer by 0.6 grade (approximately 1.9 mm., or more than one-sixteenth of an inch). The differences in both cases are highly significant, being 23 and 15 times the respective probable error.

No significant difference in length of the lint from Acala bolls that were shaded and from those that were fully exposed to the sun is indicated by the data in the lower portion of Table 5.

The coefficients of variation of lint given in Table 5 indicate no important difference in the amount of variation induced by the two kinds of pollens when applied to flowers of either variety. On the other hand, as in the case of boll period, there was much more variation in both populations of Acala bolls than in either population of Pima bolls.

## INFLUENCE OF POLLEN ON SEED FUZZINESS

The seeds of Pima cotton vary considerably in degree of fuzziness, ranging from nearly naked to entirely fuzzy. Both of these extremes are rare, however, the average condition being with about half of the seed coat covered with fuzz. In the experiment of 1928, fertilization of Pima flowers with pollen of a smoother-seeded type, Hopi, resulted in a significant decrease in the average fuzziness. As is shown by the data in Table 5, the fuzziness of the Pima seeds was increased when Pima flowers were fertilized with pollen of the much fuzzier-seeded Acala cotton. The mean difference, in comparison with seeds from fertilization with like pollen (Pima  $\times$  Pima) is less than one-half grade, but apparently significant, being 3.6 times its probable error. As has been mentioned, differences in respect to this character could not be detected in the seeds from the several populations of Acala bolls.

## DISCUSSION

It has been suggested from time to time that fertilization with different kinds of pollen may affect not only the embryo in the seed, which is the young daughter plant, but also other parts of the seed and even the fruit enveloping it, which belong to the body of the mother plant. But it remained for Swingle (9) and Nixon (7, 8) to bring forward convincing evidence that such effects occur in the date palm. They found differences in the length of time required for ripening of the fruit, in size of the fruit, and in size and shape of the seed, depending upon the kind of pollen with which fertilization was effected. The differences were most pronounced when female flowers of the date palm were fertilized with pollen of other species of Phoenix. Tissues outside the embryo and endosperm were affected, so the results could not be attributed to heterosis or to xenia, the well-known phenomenon in maize which has been accounted for as due to fertilization of the endosperm by the second generative nucleus of the pollen tube. The term "metaxenia" was invented by Swingle to designate pollen effects on the somatic tissues of the mother plant.

Evidence is presented in this paper that metaxenia occurs also in cotton, and the fact of its occurrence in the wholly unrelated groups Phoenix and Gossypium makes it probable that the phenomenon is of widespread occurrence among plants. The characters of the cotton plant in which metaxenia effects were most clearly shown—length of the lint hairs and quantity of fuzz—are both properties of the seed coat, which belongs to the body of the mother plant. The time required for development of the boll also was influenced by the kind of pollen, but in much less degree than was the time required for maturation of the date fruits in Nixon's experiments.

The metaxenia effects so far discovered in cotton parallel closely the parental relations of the characters. Thus pollen of the quickly maturing, short-linted, smooth-seeded Hopi cotton, when applied to flowers of the slowly maturing, long-linted, relatively fuzzy-seeded Pima cotton, reduced the length of the boll period, shortened the lint 7.5 per cent, and reduced the fuzz on the seeds, although the effect on the first character apparently was not significant. Conversely, pollen of the slowly maturing, long-linted Pima cotton, applied to flowers of the more rapidly maturing and shorter-linted Durango and Acala cottons, significantly increased the length of the



boll period and the length of the lint, the increase in lint length amounting to 8.2 per cent in the cross-fertilization Durango  $\times$  Pima and to 13.5 per cent in the cross-fertilization Acala  $\times$  Pima. Finally, pollen of the more rapidly maturing, shorter-linted Acala cotton, applied to flowers of Pima cotton, significantly shortened the boll period and decreased the length of the lint, the decrease in lint length amounting to 8.2 per cent.

All of these cross-fertilizations are between species belonging to very distinct groups, Pima representing the South American group, which comprises the sea-island and Egyptian cottons, while Hopi, Durango, and Acala belong to the Mexican-Central American group, which comprises the upland cottons and related forms. Cross fertilizations between two members of the latter group, Durango and Hopi, did not significantly affect the time required for development of the boll, but the length of the lint on the Durango plants was reduced significantly to the extent of 6.1 per cent by fertilization with pollen of the shorter-linted Hopi cotton.

Heterosis, or hybrid vigor, was found to be much greater in the wide crosses Pima  $\times$  Hopi, Durango  $\times$  Pima, and Pima  $\times$  Acala than in the cross between species of the same group, Durango and Hopi. The data are too scanty, however, to permit the conclusion that in cotton metaxenia effects also are greater the wider the cross. Nixon found this to be the case in Phoenix, for when flowers of the date palm (*P. dactylifera*) were fertilized with pollen of other species (*P. canariensis*, *P. roebellenii*), a more pronounced degree of metaxenia resulted than from cross fertilizations among different date palms.

The effects in these interspecies fertilizations in the genus Phoenix, unlike those in the interspecies fertilizations in *Gossypium*, were not predictable from the characters of the male parent. Thus fertilization of flowers of *Phoenix dactylifera* with pollen of *P. canariensis*, which has a very large, blunt seed, resulted in smaller and more pointed seeds than are produced by the fertilization *dactylifera*  $\times$  *dactylifera*, and the seeds resulting from the cross fertilization *dactylifera*  $\times$  *roebellenii* showed characters not seen in either of the parent species. The only analogy to the last-named effect discovered in cotton was the appearance on Durango seeds resulting from fertilization with Hopi pollen of very short lint hairs, shorter than were found on the seeds of either parent.

The nature of the mechanism involved in these metaxenia effects is unknown. Swingle and Nixon have surmised that there is an analogy to hormone action in the bodies of animals, the hypothesis being stated by Swingle (9, p. 266) as follows:

The simplest and most probable theory to explain metaxenia is that the embryo or endosperm or both of them secrete hormones, or soluble substances analogous to them, which diffuse out into the tissues of the mother plant that constitute the seed and fruit and there exert a specific effect on these tissues, varying according to the particular male parent used to fecundate the embryo and endosperm.

Since in the seeds of cotton the endosperm is but slightly developed and is absorbed at a relatively early stage, it seems likely that if substances analogous to hormones are produced they are secretions of the embryo rather than of the endosperm.

The parallelism in cotton between the metaxenia effects and the characters of the pollen parent suggests the hypothesis that secretions

may be initiated by the genes which determine the characters, but the absence of such parallelism in Phoenix is an obstacle to the generalization of this idea.

The demonstration of metaxenia in cotton is of practical interest in relation to uniformity of lint length, a very important property. It has been shown that fertilization of Pima cotton flowers with pollen of the shorter-linted Hopi and Acala cottons shortened the lint on the Pima plants by more than one-sixteenth of an inch as compared with the lint produced by flowers fertilized with Pima pollen. Fertilization of Durango and of Acala upland flowers with pollen of the long-linted Pima cotton, on the other hand, lengthened the lint approximately one-sixteenth of an inch as compared with the lint from flowers fertilized with like pollen. Such differences in length are not negligible from the spinner's point of view.

Wherever two varieties of cotton are grown in neighboring fields, more or less transfer of pollen by bees and other insects occurs. Only a small percentage of the ovules in a field are likely to be cross-fertilized in this way, but if the two varieties differ greatly in staple such crossing as takes place will be detrimental to the uniformity of the immediate product as well as to the value of the seed for future planting. A certain proportion of the lint will be shorter than the average in the field of long-staple cotton, and longer than the average in the field of short-staple cotton. The discovery of metaxenia in respect to lint length therefore gives an additional reason for advocating the growing of only one kind of cotton in a neighborhood.

#### SUMMARY

Experiments to determine whether metaxenia, or immediate effects of pollen on tissues of the mother plant, occurs in cotton were suggested by the discovery of this phenomenon in the date palm by Swingle and Nixon.

The first experiment involved two different types of cotton, Pima Egyptian and Hopi. These forms represent the two main groups of New-World cottons, the Pima belonging to the South American group, and Hopi to the Mexican-Central American group.

Flowers on some of the Pima plants were fertilized with Hopi pollen, while flowers on other Pima plants were left to natural pollination.

The boll period, or number of days from anthesis to opening of the boll, was computed from a record of each individual boll. Determinations of length of lint, lint index, seed fuzziness, number of seeds per boll, and weight of the individual seeds were made on approximately 100 bolls from each fertilization. The statistical constants of the six characters are given in Table 1, and the coefficients of correlation among them (excepting boll period) are given in Table 2.

A mean difference of 18 days between the boll periods of the parent types was observed. The boll period on Pima plants averaged 1.1 days shorter from flowers fertilized with Hopi pollen than from flowers fertilized with Pima pollen. Environment is believed to have neutralized partly the effects of unlike pollen.

The lint lengths (fig. 2) of Pima and Hopi average about  $1\frac{1}{8}$  inches and seven-eighths of an inch, respectively. A significant reduction in length of lint of a full one-sixteenth of an inch resulted from fertilization of Pima flowers with Hopi pollen. Consideration of the cor-

relations of lint length with the other characters studied makes metaxenia a reasonable explanation of this difference.

Pima and Hopi differ greatly in abundance of lint on the seeds (fig. 2), but the means for lint index of seeds on Pima plants resulting from fertilization with Pima and with Hopi pollens, respectively, did not differ significantly.

When flowers of the relatively fuzzy-seeded Pima family were fertilized with pollen from the nearly naked-seeded Hopi family (fig. 3), a significant reduction in fuzziness resulted. Correlations of seed fuzziness with other characters in these populations do not invalidate the conclusion that this difference also is a metaxenia effect.

The mean weight of the individual seed from the fertilization Pima  $\times$  Hopi was significantly greater than from the cross-fertilization Pima  $\times$  Pima, but this difference is attributed to heterosis rather than to metaxenia.

A second experiment involved Pima Egyptian, Durango upland, and Hopi. The length of lint of Pima averages  $1\frac{5}{8}$  inches, of Durango  $1\frac{1}{4}$  inches, and of Hopi seven-eighths of an inch. (Fig. 2.) The boll period of Durango averages longer than that of Hopi but much shorter than that of Pima.

Durango, because of its intermediate lint length, was used as the female parent. Approximately equal numbers of flowers were pollinated daily with Pima, Durango, and Hopi pollens. A rotation was followed that resulted in approximately equal numbers of bolls from each of the three fertilizations on each of the plants and equalized individual plant differences and seasonal variations.

The characters studied were the same as in the first experiment except seed fuzziness, which was disregarded because of the extreme fuzziness of Durango seeds. (Fig. 3.) The statistical constants of the characters determined on 100 bolls from each fertilization are given in Table 3, and the coefficients of correlation among them are given in Table 4.

The mean boll period on Durango plants was slightly but significantly increased by fertilization with Pima pollen as compared with fertilization by Durango and by Hopi pollen. Since there was no correlation of boll period with other characters, a metaxenia effect is clearly shown.

Pollen of the longer-linted Pima variety lengthened the lint of the intermediate Durango variety, and pollen of the shorter-linted Hopi variety shortened it. The absence of correlation of lint length with other characters determined leaves metaxenia as the only satisfactory explanation of the differences observed.

Very short lint hairs (fig. 4) on Durango seeds resulting from fertilization with Hopi pollen was the only extra-parental character showing analogy to those discovered by Nixon in cross-fertilizations between different species of *Phoenix*.

Fertilization of Durango flowers with Pima pollen resulted in a higher mean lint index than fertilization with Durango and with Hopi pollens. The differences are scarcely significant and are probably attributable to heterosis, since lint index was positively correlated with seed weight in all three populations.

A heterosis effect on weight of seeds analogous to that shown by the  $F_1$  plants of hybrids between upland and Egyptian was observed in the cross-fertilization Durango  $\times$  Pima as compared with the cross-

fertilization Durango  $\times$  Durango. Cross-fertilization between the closely related forms Durango and Hopi did not affect weight of seeds.

Almost as great a metaxenia effect on lint length of Durango upland cotton was produced by Hopi pollen as by Pima pollen, although the hybrid plants show pronounced heterosis even in the embryonic stage in crosses between upland and Pima cottons and very little, or none, in crosses between upland and Hopi cottons.

Reciprocal cross-pollinations of Pima Egyptian and Acala upland cottons, the varieties most extensively grown in the Salt River Valley, Ariz., gave results in agreement with those of the earlier experiments. The time required for maturation of the boll was significantly decreased when Pima flowers were fertilized with Acala pollen and was significantly increased when Acala flowers were fertilized with Pima pollen. The lint length was significantly decreased when Pima flowers were fertilized with Acala pollen and significantly increased when Acala flowers were fertilized with Pima pollen. In both cases the difference, in comparison with the lint length from fertilization with like pollen, averaged slightly more than one-sixteenth of an inch. The fuzziness of the seeds on the Pima plants was slightly but significantly greater when the flowers were fertilized with Acala pollen.

The metaxenia effects in cotton have been observed in the length of time required for the fruit to mature, in length of lint, and in quantity of fuzz on the seeds. The effect on the first character has been observed in a much more pronounced degree in the date palm. Lint length and fuzziness are properties of the outer seed coat, which is part of the body of the mother plant.

Contrary to the discoveries by Nixon in the genus *Phoenix*, the metaxenia effects disclosed in cross-fertilizations between different species of cotton parallel the expression of the characters in the parent species.

The theory advanced by Swingle, which attributes metaxenia in *Phoenix* to a hormonelike action of substances secreted by the embryo or the endosperm, seems to account equally well for the results obtained in *Gossypium*.

The metaxenia effect on length of lint of cotton suggests the danger of growing two or more varieties of widely divergent staple lengths in the same vicinity, as the uniformity of both products is likely to be impaired to the extent that cross-fertilization occurs.

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# DEVELOPMENT OF THE CITRUS-SCAB ORGANISM, *SPHACELOMA FAWCETTII*<sup>1</sup>

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## INTRODUCTION

During February and the spring of 1925, field observations were made by the writer on characteristics of the citrus-scab organism (*Sphaceloma fawcettii* Jenkins) (9)<sup>3</sup> as developed on citrus in the rutaceous collection at the United States Citrus Disease Field Laboratory at Orlando, Fla. Based on these studies, the general aspect of the disease, particularly as affecting leaves of citrus, was later outlined by the writer, as reported by Fawcett and Lee (7, p. 223-226,<sup>4</sup> 487, and fig. 63).

In September, 1928, additional observations were made in the same place, as well as in the collection at the Florida Agricultural Experiment Station at Gainesville. The present paper embodies the data thus obtained, especially those based upon the superficial development of the fungus in Florida on spring and autumn growth of leaves of sour orange (*Citrus aurantium* L.) and on young fruit of grapefruit (*C. grandis* Osbeck), as shown in Plate 1 and Figures 1 and 2, and on similar leaf growth of grapefruit and Tahiti lime (*C. aurantifolia* Sw.). Secondary fungi associated with the fungus are mentioned, and data on cultural studies and inoculation tests are included.

The main purpose of the present paper is to present data relative to citrus scab and its pathogene that will aid in distinguishing them from other diseases and organisms with which they have been confused. For this reason data such as those pertaining to the coloration of the fungus are given in considerable detail. Except in the historical section, no reference is made to scab in other citrus-growing countries, as the writer hopes to present later an account of comparative studies of the pathogene in this and other countries.

## HISTORICAL REVIEW

The first published report of the occurrence of *Sphaceloma fawcettii* in Florida, in other citrus-growing regions of the United States, or

<sup>1</sup> Received for publication Nov. 25, 1930; issued May, 1931. Most of the data presented in this paper, of which the title was previously published (11)<sup>3</sup> as "Certain Characteristics of the Citrus-Scab Organism," are part of a thesis presented to the faculty of the Graduate School of Cornell University in June, 1927, in partial fulfillment of the requirements for the degree of doctor of philosophy.

<sup>2</sup> Thanks are due H. M. Fitzpatrick, L. M. Massey, and A. J. Eames, under whose direction the work was done. The writer also appreciates the assistance rendered by H. R. Fulton, F. W. Pennell, T. R. Robinson, H. E. Stevens, G. F. Weber, Erdman West, O. E. F. Winberg, J. R. Winston, and others in obtaining some of the material on which the studies were based, as well as that rendered by H. S. Fawcett in various other ways. The work was done in cooperation with the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and with the Department of Plant Pathology at Cornell University and at the University of Florida. Color drawings are by J. Marion Shull, and photographs and photomicrographs by Marcel L. F. Foubert, with the exception of those in Figure 4, C and D, which are by J. F. Brewer.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 557.

<sup>4</sup> In the account reported by Fawcett and Lee the next to the last sentence concluding the first paragraph should read as follows: "Old lesions on grapefruit often remain pink or rose colored, presenting a smooth scarred appearance."

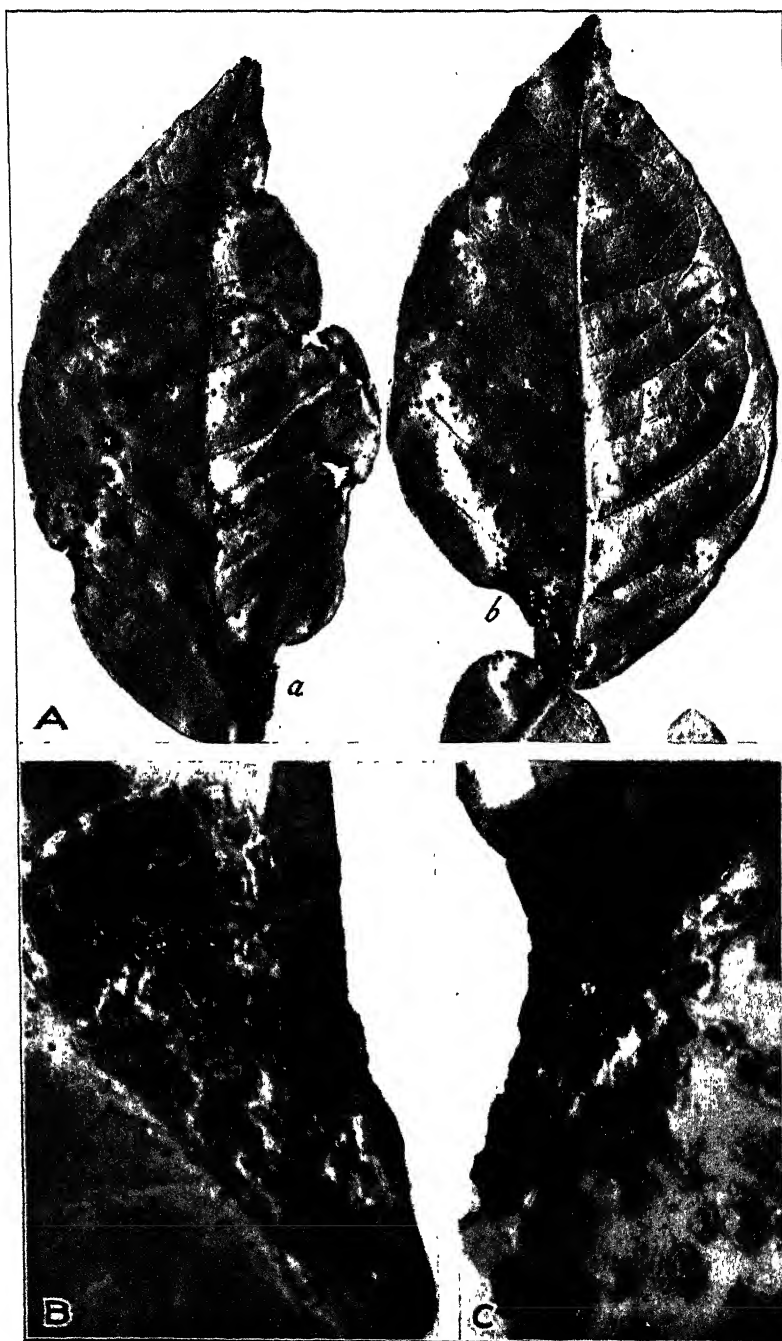


FIGURE 1.—A, Abundant dark conical fructifications of *Sphaceloma fauettii* on lower surface of fairly young sour-orange leaves.  $\times 1$ . B, Enlargement of A, a.  $\times 6\frac{1}{2}$ . C, Enlargement of A, b.  $\times 6\frac{1}{2}$ . Material from Gainesville, Fla., September, 1928

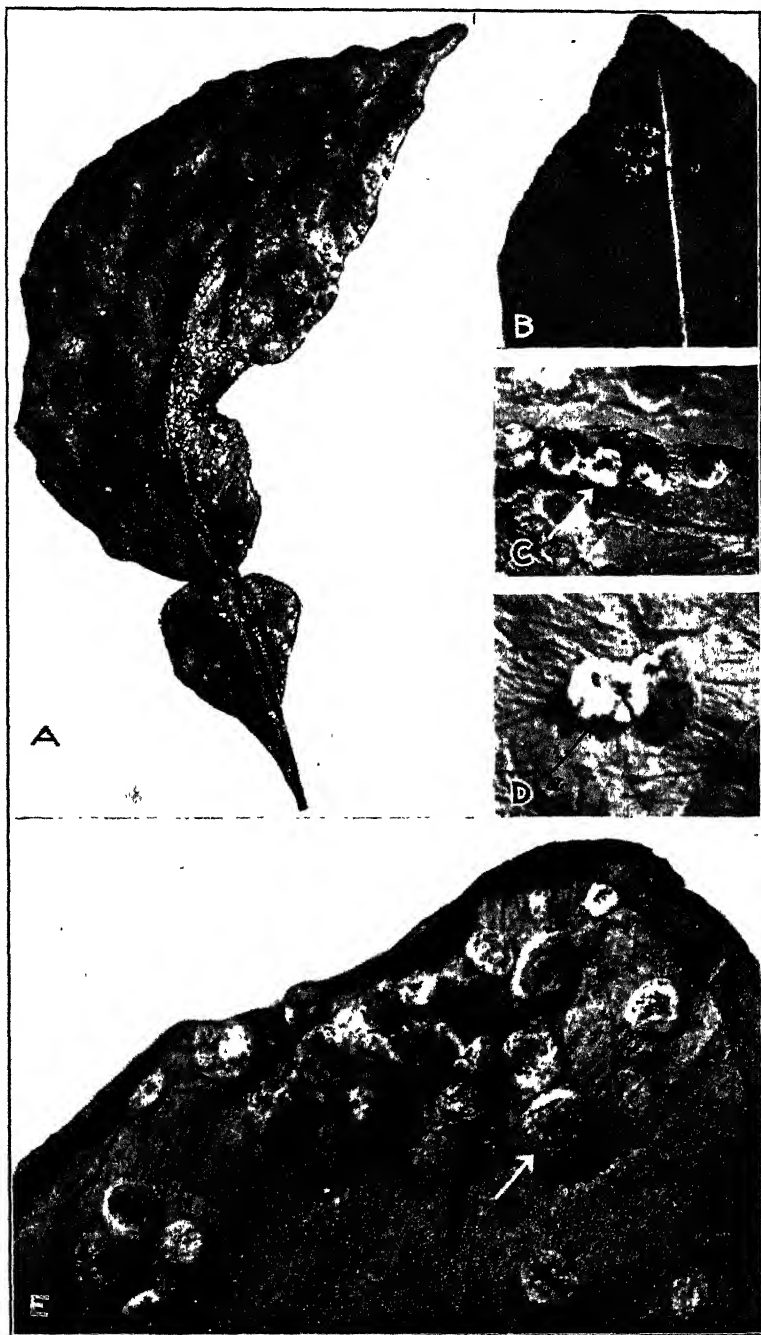


FIGURE 2.—*Sphaceloma fawcettii* on lower side of 2-week-old scabbed sour-orange leaves. A, White or pale masses of conidiophores and conidia.  $\times 1$ . B, a, Dark-colored masses of conidiophores and conidia.  $\times 1$ . C, a, and D, a, Secondary *Fusarium*.  $\times 6\frac{1}{2}$ . E, Isolated groups of conidial fructifications, some in circular arrangement (a) on rims of prominent galls.  $\times 6\frac{1}{2}$ . Material from Gainesville, Fla., September, 1928



even in the Western Hemisphere, appears to be that by Scribner (17), dated August, 1886. The account is based upon specimens or information transmitted to the United States Department of Agriculture from Ocala and Deland, Fla., during 1885 and 1886. Swingle and Webber (21) later noted that the disease had been recognized in Florida "in about the year 1884." W. T. Swingle has recently informed the writer that this date is based upon the observation of scab at that time in a nursery near Lake Weir, afterwards visited by himself and H. J. Webber. From examinations of specimens from Florida and Paraguay recently identified by the writer and now inserted in the Mycological Collections of the Bureau of Plant Industry, it is evident that the organism was established on citrus in both North America and South America at an even earlier date. The specimen from Florida, labeled only "May 1, 1878, Fla.," was originally from a collection of fungi owned by the late W. H. Seaman. The more complete information on the label of the South American specimen reads as follows: "B. Balansa—Pl. du Paraguay. 1878-1884. No. 3543. Ecorces d'oranges douces. Villa Rica, 13 janvier 1882." On this specimen, although acervuli were numerous, no conidia were seen, so that until additional data are available the specific identification of the fungus as *S. fawcettii* should perhaps be regarded as not absolutely positive. The circular arrangement of scab lesions on leaves of citrus (fig. 2, B) grown in Paraguay, noted by Spegazzini (18), corresponds to that on such growth developed in Florida and later described by the writer, as reported by Fawcett and Lee (7, p. 225).

Scribner (17) thought that *Sphaceloma fawcettii* was of recent occurrence in Florida. Swingle and Webber (21) expressed the opinion that it had been introduced on Satsuma (*Citrus nobilis unshiu* Sw.) from Japan, from which country the first importation of this citrus variety was brought to Florida in 1876 (1). As identified by W. T. Swingle for the writer, the kind of citrus represented by the Seaman specimen is probably sour orange, which is the same species as that on which the Satsuma was first budded in this country (1). It is of interest to note that the fungus is not present on material of sour orange and other citrus varieties, not including Satsuma, contained in the Schweinitz herbarium, as acquired by the Academy of Natural Sciences, Philadelphia, in 1834. These specimens bear no date.

Comparisons of *Sphaceloma fawcettii* with the similar species of *Sphaceloma* causing the disease of avocado (*Persea spp.*) known as avocado scab, made in 1925 by the writer (10), revealed that these two organisms had formerly been confused (19, 20, 25) and that the one from citrus is not pathogenic on avocado, or at least not on some varieties, as heretofore supposed (19, 20). On the basis of information then in part unpublished, the writer (9) had formerly assumed that the two organisms or diseases were identical.

Scribner (17) reported that sour orange and lemon (*Citrus limonia* Osbeck) were particularly susceptible to citrus scab, but that the sweet orange (*C. sinensis* Osbeck), even now regarded as immune or highly resistant (25, 26,) was not affected. Swingle and Webber (21) commented upon the economic importance of scab in the lemon groves of Florida, and more recently Winston (25) has explained that to this disease was largely due the "failure of the lemon industry in" that State, which before the fungus became established there

"gave promise of becoming a very profitable undertaking." Many rutaceous hosts other than those named in the present paper are known to be susceptible to attack by *Sphaceloma fawcettii*, or by what, so far as known, is this species (25, 26). This fungus, or at least a *Sphaceloma* on citrus, is firmly established in all citrus-growing regions where climatic conditions favor its development (6, 13, 25). Peltier and Frederick (13) do not include Java among such regions, but scabbed citrus at hand, collected at Buitenzorg in 1919 by R. D. Rands, leaves no doubt of the occurrence there of a *Sphaceloma* on citrus. Whether this organism is identical with *S. fawcettii* remains to be determined.

## FRUCTIFICATIONS ON SCAB LESIONS

### DISTRIBUTION

On young scab lesions found on leaves the fungus generally fruits in abundance on the surface originally infected. It was with reference to this surface that the writer made the statement (7, p. 224) that the central, often craterlike area of young galls (fig. 2, B, a), i. e., the type of lesion whose histologic structure is described by Cunningham (3) and Butler (2), is covered with a fine velvety layer of conidiophores and conidia, which slowly disappears with the aging of the leaf. As concerns young lesions of the gall type, particularly those considerably raised, subsequent observations have revealed that such surfaces from the first may be practically free from fructifications of the fungus; or that the fructifications may occur only in small solitary clumps or larger masses on the marginal regions or rims (fig. 2, E), sometimes outlining troughlike depressions or grooves (figs. 2, E, a, and 3, A, a). Where a layer of phellem extends as a continuous band from one side of the leaf to the other, the fungus sometimes fruits on both surfaces of the intervening region of the lesion, although in greater abundance on the surface originally infected. Where the necrotic centers of such lesions have fallen away, the fungus may fruit scantily on the faces of the hypertrophied tissue thereby exposed. On lesions in which the tissue has become completely necrotic (fig. 1, A, b, and C), possibly owing to the absence of a well-developed phellem, conidial fructifications often cover practically the entire area of the surface originally infected (fig. 3, B), and may also be numerous on the opposite side (fig. 3, D). The necrotic lesion shown in Figure 1, A, b, and C, of which a section is shown in Figure 3, D, is a compound lesion; while that illustrated in Figure 3, B, as in the case of the gall type of lesion shown in Figure 3, A, probably resulted from an individual infection. Fructifications of the fungus were present only on the lower side of the compound lesion of the gall type illustrated in Figure 1, A, a, and B; that is, on the surface there represented.

At Orlando, Fla., during the spring of 1925, a record was made of the persistence of conidial fructifications of *Sphaceloma fawcettii* on living scabbed leaves of sour orange. The record showed that they may be present on the same lesions for as many as four months.

### COLORATION

Masses of conidia and conidiophores on the surface of the lesions on young fresh leaves, stems, and fruits of citrus, as observed at

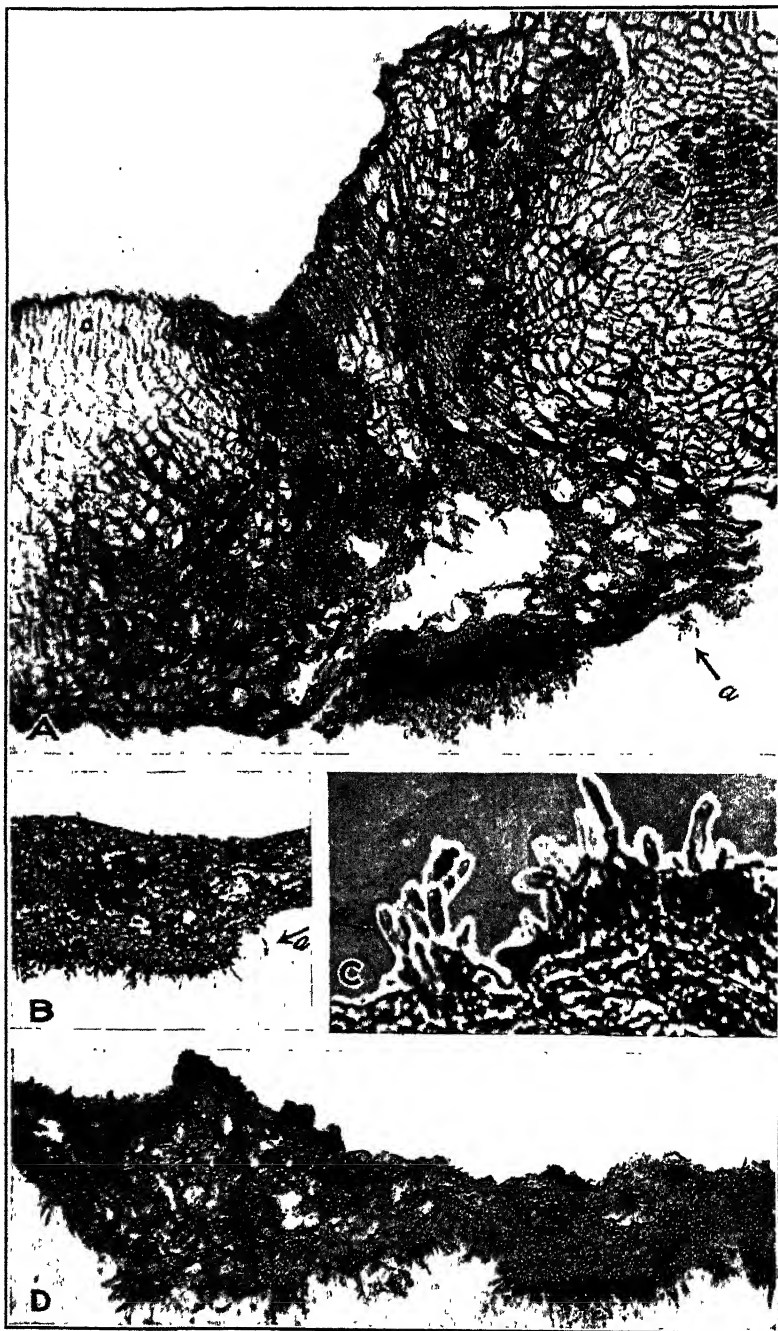
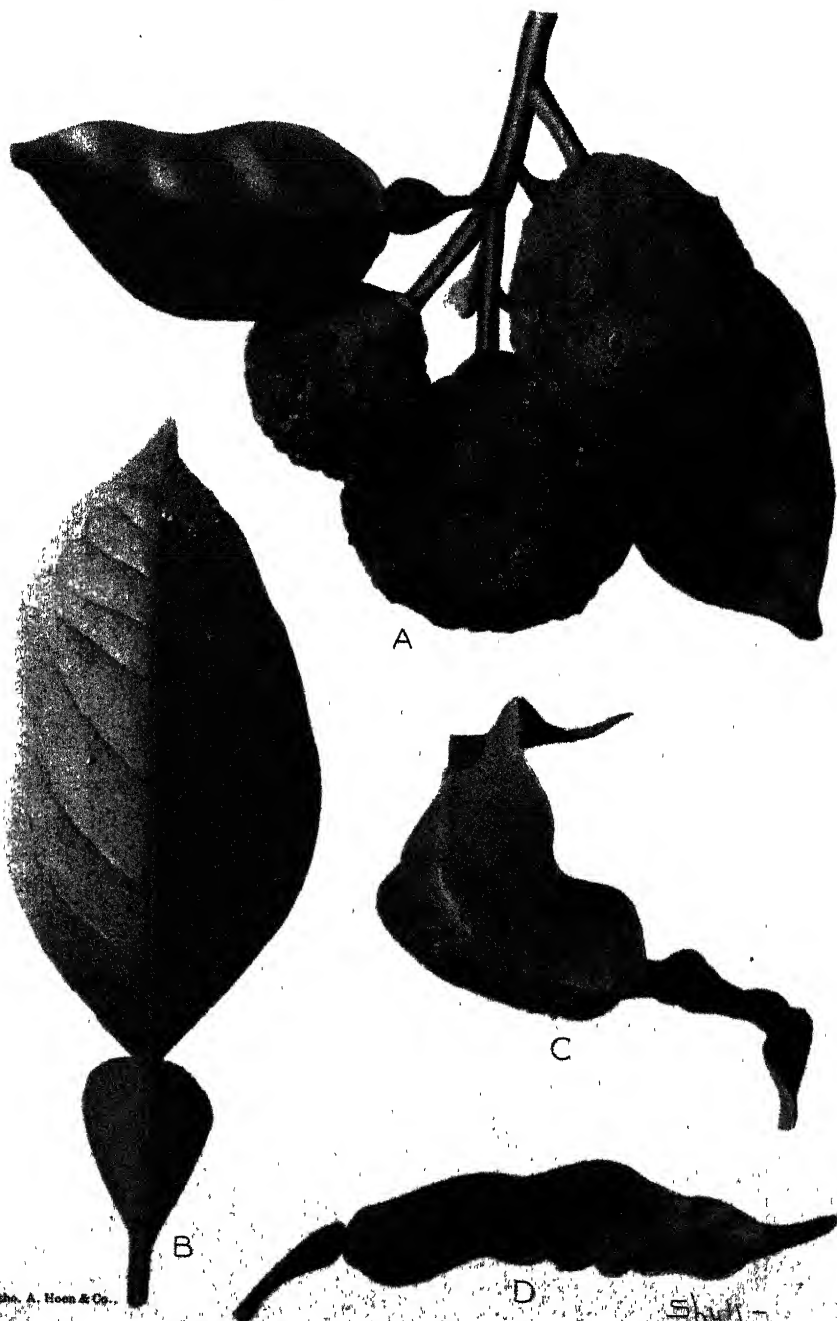


FIGURE 3.—Cross sections through scabbed leaf lesions. A, Large sporodochium on concavity on lower surface of gall; *a*, solitary clump of conidiophores and conidia.  $\times$  about 200. B, Fructifications covering thickened region of necrotic lesion. C, Enlargement of peripheral region of B, showing more clearly (*a*) clump of conidiophores bearing conidia there labeled *a*. B,  $\times$  100; C,  $\times$  about 600. D, Fructifications on compound necrotic lesion, covering not only the entire lower surface but also part of the upper.  $\times$  about 200. Material from Gainesville, Fla., September, 1928





A, scab lesions on young fruits of grapefruit; B-D, scab lesions on lower side of several weeks-old leaves of sour orange, confidial fructifications of the causal fungus visible only on the confluent lesion shown in C. X1. Material from Orlando, Fla., in the spring of 1925

Orlando during the spring of 1925, were usually drab<sup>5</sup> in color; on leaves approximately 2 months old they ranged from drab to vinaceous drab or dark olive-gray (7, p. 224-225).<sup>6</sup> Other colorations of the fungus observed in 1925 are Saccardo's umber, pinkish cinnamon, fuscous, and a brownish color termed "hair brown." Hair brown represented the color of the fungus on a single leaf of grapefruit fully 2 months old, and fuscous its development on succulent leaves not more than 2 weeks old. The older leaf was taken from a large tree and the younger ones from small nursery plants. Of the numerous color readings made at Orlando in 1925, these particular colorings were recorded only once. On young scabbed citrus observed at Orlando in the autumn of 1928 the *Sphaceloma* cover on the surface of the lesions, as well as the lesions themselves, was of essentially the same appearance as in the spring of 1925. None of the fuscous-colored growth was observed. At Gainesville, however, this was the prevailing color of conidial fructifications of the fungus on young sour-orange leaves (figs. 1 and 2, B and E), although occasionally they were pure white or just becoming colored (fig. 2, A). Contrasting sharply with the warm bright hues of the lesions or the immediately surrounding green healthy leaf surface, the fructifications were most noticeable when fuscous in color. (Figs. 1 and 2, B.) In their drab or nearly drab coloration (pl. 1, C), however, they were rather delicate in appearance and, except as seen through a hand lens, often were practically obscured by the warm hues of the lesions themselves (pl. 1, A, B, and D).

#### MICROSCOPIC APPEARANCE AND DEVELOPMENT

##### CONIDIOPHORES

Many of the small, compact acervuli of *Sphaceloma fawcettii*, often enlarging to form sporodochia (fig. 3, A and D), were originally confined to a single cell or a few epidermal cells. Their early development was similar to that previously illustrated by the writer (9, fig. 3) for this fungus as produced in culture and here represented in Figures 4, A, and 3, C, *a*. The latter illustration shows the formation of conidiophores in the leaf tissue, but whether they are beneath the epidermis or beneath only the cuticle is not clear. At an earlier stage in its development the acervulus shown in Figure 4, A, *b*, probably resembled that represented in Figure 4, A, *c*; both apparently arose from horizontal hyphae like those faintly shown in Figure 4, A, *a*. Original clumps of conidiophores were often distinct, even after continued growth from them had taken place. (Fig. 4, E and G.) As in culture (9, fig. 3), when first produced on the citrus substrate the conidiophores were mostly continuous or 1-septate (fig. 4, A, *b*). They were sometimes colored even before being exposed; often they seemed to be firmly held together, or embedded, in a clear hardened substance apparently derived from their walls. Where conidiophores had continued to grow, by the formation of conidia remaining in situ or by further hyphal growth, they were ordinarily not more than 25 or 30  $\mu$  long, suggesting those of *Cladosporium* or *Fusicladium*. In the unusually large sporodochial development on the gall type of lesion represented in Figure 3, A, the longest conidiophores measured approximately 100  $\mu$ . Some of those composing

<sup>5</sup> RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

<sup>6</sup> Color readings, based on Ridgway's color guide, are by J. Marion Shull, F. R. Cole, and the writer.

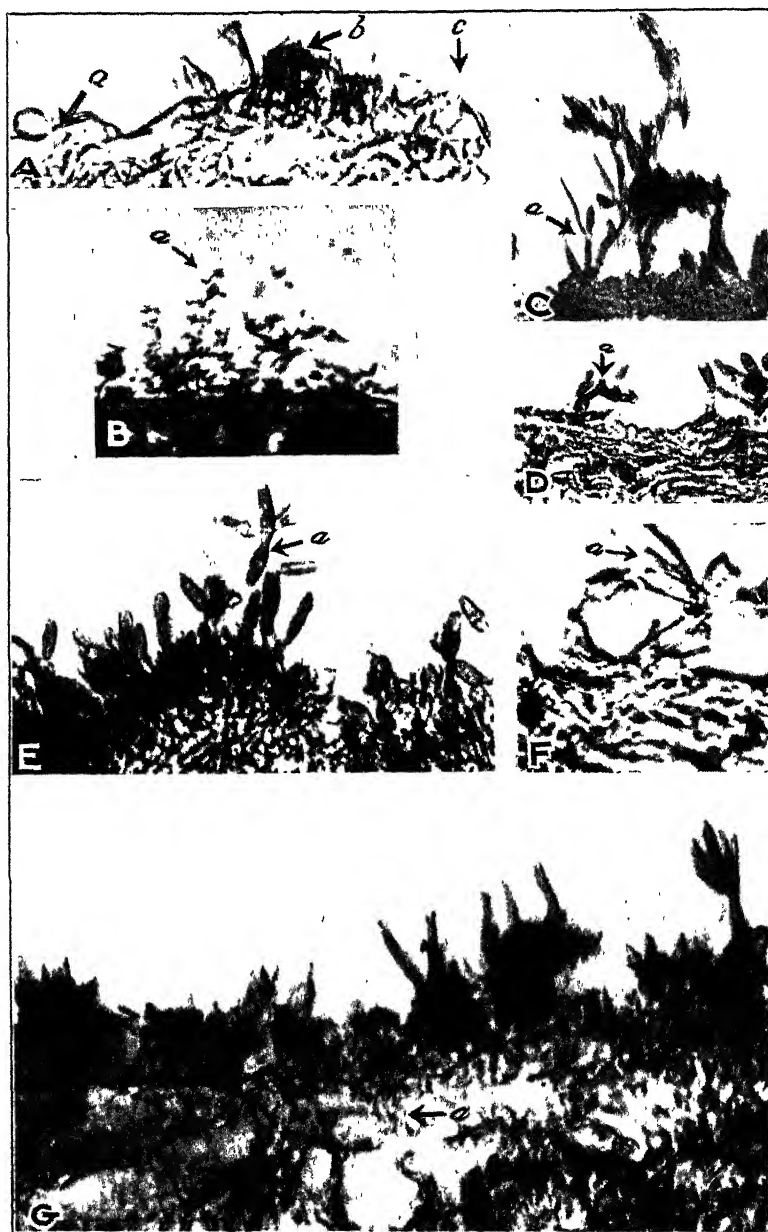


FIGURE 4.—Parts of cross sections through necrotic lesions. A, *a*, Hyaline hyphae; *b* and *c*, acervuli.  $\times$  about 600. B, *a*, Small spherical conidia produced on conidiophores.  $\times$  about 600. C, *a*, A conidiophore which first produced a conidium and then continued its growth as a hypha instead of producing another conidium.  $\times$  400. D, *a*, Septate elongate conidium which has produced a secondary conidium while still attached to conidiophore.  $\times$  400. E, Enlargement of part of section shown in Fig. 3, D; *a*, conidium in situ from which several secondary conidia have developed.  $\times$  about 600. F, *a*, Group of spindle-shaped conidia partially dislodged from conidiophores.  $\times$  about 600. G, Small clumps of conidiophores one of which bears a cluster of conidia at its apex; *a*, hyphae penetrating the leaf structure.  $\times$  about 600. Material from Gainesville, Fla., September, 1928

the pale cottony growth illustrated in Figure 2, A, attained twice this length. Dark conidiophores of more usual length often arose from the entire surface of thick pale-colored stromata, covering the central areas of lesions of the gall type or extending for a considerable distance on the surface of lesions entirely necrotic. Figure 4, C, *a*, represents a conidiophore which first produced a conidium and then continued its growth as a hypha instead of producing another conidium.

#### CONIDIAL FORMS

Conidia in preparations made from the *Sphaceloma* cover on scab lesions consisted of (1) ovoid elliptical hyaline conidia, (2) ovoid elliptical (fig. 3, C, *a*) or spindle-shaped (fig. 4, F, *a*) colored conidia, and (3) small spherical conidia (fig. 4, B, *a*) not previously reported. These three types were considered to be homologous or merely to represent older or younger forms. The colored conidia ranged from pale yellowish to reddish brown or nearly black. At times it could be discerned that their dark coloration was limited to a hardened outer wall or epispore. Occasionally this was irregularly thickened or roughened, although ordinarily it was smooth. Where the epispore of dark-colored elongate conidia had become ruptured the hyaline endoconidia thus exposed were indistinguishable from newly formed elongate hyaline conidia such as have been previously illustrated by the writer (9, figs. 3 and 4).

The elongate colored conidia, which were present in great numbers, commonly measured about 10 by 4  $\mu$ , although occasionally they reached 16  $\mu$  in length. They were usually continuous but sometimes 1-septate or, more rarely, 2-septate. (Figs. 3, C, *a*, and 4, E-G). In the 1-septate type a number were seen to be breaking apart at the septum or to have become entirely separated. They were sometimes produced on the sides of the conidiophores, or what were originally conidia, all or part of the way from the base to the apex, extending outward more or less at right angles. (Fig. 4, E, *a*.) The septate structure shown in Figure 4, D, *a*, appears to be a primary conidium continuing its growth in situ on the conidiophore on which it was borne; a secondary conidium has been produced.

Elongate hyaline conidia were usually seen in preparations made during periods of precipitation or when the leaves were wet with dew. Usually detached, many of them had sprouted from colored elongate conidia of which they were only younger forms; they were usually produced at the end or perhaps even more commonly at a point just to the side, as is illustrated by Petch (14, figs. 3 and 4) for what appears to be a comparable mode of conidial formation in *Myriangium duriaei* Mont. and B., not hitherto interpreted as such. They were formed in the same manner in from 1 to 3 hours when the elongate, colored conidia were sown in drops of water on glass slides.

The spherical microconidia were seen on numerous occasions at Orlando, Fla., during the spring of 1925. They ranged from 1 to 4  $\mu$  in diameter. Those of the hyaline type were often glistening or highly refringent, particularly when thick-walled. As in the case of the elongate conidia, they sometimes budded from each other. On a few occasions they were produced from the surfaces of elongate colored conidia sown on corn-meal agar medium. The masses of glistening or refringent bodies embedded in the free mucilaginous



substance previously reported as present in this fungus (7, p. 490) were probably masses of microconidia. In one instance small glistening microconidia, about the size of those shown in Figure 4, a, were seen grouped about the pointed apexes of conidiophores still contained within an unruptured epidermal cell. Possibly under more favorable circumstances the larger elongate conidia would have formed. Assuming that the refringent granules observed in *Sphaceloma ampelinum* DBy. by Prilleux (15, p. 316; 16, p. 37-38), by Viala (22, p. 304-305, fig. 119), and by Viala and Pacottet (23, p. 663; 24, p. 89, fig. 22) were all microconidia, those of *S. fawcettii* would, of course, correspond to them, as in the case of those produced by *S. symphoricarpi* Barrus and Horsfall (12).

The comparatively few swollen or germinated conidia seen on the surface of scab lesions corresponded to those formerly illustrated as developed in culture from elongate hyaline conidia (9). Elongate colored conidia that had become greatly swollen and 1-septate or muriform suggested forms referred to *Coniothecium* by Guéguen (8, pl. 10). Spherical hyaline bodies, visible through the walls of one of them, were probably homologous to the sporelike bodies observed in culture by Fawcett (5) within what was probably the original cell of germinated hyaline conidia 3 days old. Several hundred small conidia of various sizes, present in the vicinity of an elongate colored conidium that had germinated, apparently had been produced from its surface.

#### ISOLATIONS AND CULTURES

Sixteen or more isolations of *Sphaceloma fawcettii* from scabbed citrus grown in Florida were made by the writer during 1925 and 1926. Most of them consisted of dilution-plate cultures from the elongate colored conidia on bittersweet orange, sour orange, grapefruit, Satsuma, Tahiti lime, calamondin (*Citrus mitis* Blanco), and a lemon hybrid. A single set of isolation cultures often produced hundreds of small colonies characteristic of those previously described for this organism (4; 5; 7, p. 490; 25). In order to compare them in parallel, a culture from practically every set of isolations was preserved. The inoculum for such stock cultures ordinarily consisted of a single isolated colony; in most cases this probably represented growth from a single conidium. One culture from Tahiti lime (culture 71), was definitely known to have developed from a single conidium. These cultures and culture 8, which is the single conidium strain of the Stevens culture isolated in 1916 (9), were compared in parallel on test-tube slants of carrot, Molisch's, potato-dextrose, and other agar media. The inoculum here consisted of a mass of stromatic growth about 5 mm. in diameter transferred from each stock culture. In common with culture 8, on the three media named most of the cultures produced a convoluted type of colony. (Fig. 5, A.) Culture 71, however, proved to be decidedly pulvinate as compared with culture 8 or with most of the other isolations that have been made to date. (Fig. 5, B.) Its coloration also was somewhat different; for example, compared in parallel with culture 8, in a certain set of 17-day-old cultures on carrot agar, it was vinaceous brown, ranging lighter and darker; whereas culture 8 was at the same time Tilleul buff. An isolation from bittersweet orange showed features intermediate between those of cultures 8 and 71, in being pulvinate or hemispherical

on Molisch's medium, even when several weeks old (fig. 5, C) and definitely convoluted on carrot-agar medium (fig. 5 D.)

The culture from the bittersweet orange is no longer alive, but cultures 8 and 71 are still in the stock-culture collection. Although they have been transferred many times, their gross cultural characteristics still remain distinct as described. Such marked cultural variations are of course to be taken into consideration when comparisons are based upon gross cultural characteristics. Their significance otherwise is not understood. It may be noted, however, not only that corresponding cultural differences have been observed by the writer for some of the species of *Sphaceloma* listed in a previous publication (12, p. 46-47), but also, judging by Petch's (14) illustrations, that similar types of growth occur in *Myriangium* as developed on the host plant. Fawcett has informed the writer recently that in *Sphaceloma fawcettii* he has observed the pulvinate as well as the convolute type of growth here described.

Culture 8, in 1-week-old cultures on potato-dextrose agar slants held for a week at constantly maintained temperatures of 0°, 7.5°, 10°, 15°, and 20° C., had produced none or only a little growth at 5°, and slight growth at 7.5°. A mucous covering, like that shown in Figure 5, C, was present on all of the cultures grown at these temperatures, being most abundant in the cultures held at 10° and 15°. Practically the same results were obtained with isolations of a number of other *Sphaceloma* fungi grown in parallel cultures with culture 8. In the cultures of *S. ampelinum*, causing anthracnose of grape (*Vitis*), it was produced in a noticeably larger quantity than in any of the others, appearing as a thick, clear, glistening mass. After 12 days at a temperature of 20°, the mucous covering was still present in only this one species. At any one temperature all of the different isolations grew at about the same rate.

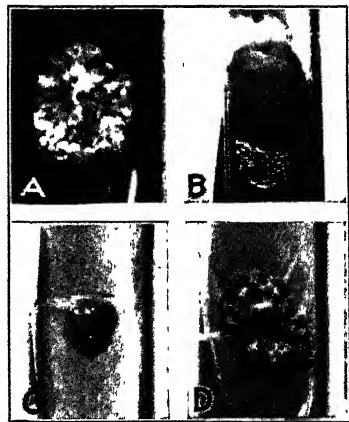


FIGURE 5.—*Sphaceloma fawcettii* on agar media. Convoluted (A) and pulvinate (B) types of colonies represented by two different isolations grown on the same medium, potato-dextrose, and by a third isolation (C and D), grown on two different agar media, Molisch's (C) and carrot (D).  
× 1

#### ASSOCIATED FUNGI

A *Fusarium*, identified by C. D. Sherbakoff as *F. fructigenum* Fr., was observed both at Orlando and at Gainesville, Fla., as forming fluffy pinkish borders about the scab lesions or galls, often covering all but the central area occupied by the *Sphaceloma*. (Fig. 2, C, a, and D, a.) This may be the *Fusarium* that J. B. Ellis found on early specimens of scabbed citrus from Ocala, Fla., sent him by Scribner, and which Ellis thought might be the cause of the disease (17, p. 182). Two other secondary fungi occasionally seen on scab lesions are *Colletotrichum gloeosporioides* Penz. and a pycnidial fungus which is possibly a species of *Phoma*. Superficially both resembled dark acervuli of *Sphaceloma fawcettii* such as are shown on the upper

surface of the section illustrated in Figure 3, D. The first secondary fungus grew on the upper side of necrotic leaf lesions and the second on the gall type of lesion on young stems. At Orlando, on scab lesions on an old lemon fruit still hanging on the tree, were coarse conidial fructifications identical with or resembling those usually referred to *Cladosporium herbarum* Massee. It was not determined whether they were identical with a *Cladosporium* common on the Orlando citrus collection, which sometimes developed in original isolation cultures of *S. fawcettii*.

The *Cladosporium* referred to previously as occasionally developing in original isolation cultures of *Sphaceloma fawcettii* was readily distinguishable from this organism. Rather delicate as seen in culture, its pale grayish colonies developed much faster than those of *S. fawcettii* and were noticeably less stromatic. The different types of conidia produced by the *Cladosporium* in culture were sometimes indistinguishable from those of *S. fawcettii* as developed on citrus in the rutaceous collection. The usual absence of conidial fructifications in cultures of *S. fawcettii* has been noted previously (25). This similarity included not only size, color, and general form, but also methods of conidial formation, among them the prompt development of hyaline oval or elliptical biguttulate conidia when the elongate colored conidia were transferred from a less humid to a more moist environment. Although generally longer than the *Cladosporium*-like conidiophores of *S. fawcettii*, they were of about the same width.

#### INOCULATIONS

Through inoculation tests the pathogenicity of culture 8 on lemon was proved in 1924 (9). In parallel inoculations with other *Sphaceloma* isolations representing the same or other species, this culture has since been employed in inoculation tests on four other kinds of citrus, namely, rough lemon (*Citrus limonia*, No. 7610<sup>7</sup>), grapefruit, Thomasville citrangequat (*Fortunella marginata* × Willits citrange, No. 48010), and Cuban shaddock (*Citrus* sp. hybrid, No. 11893). The rough lemon was inoculated in 1926, the others in 1927. The inoculations were made in greenhouses at the Arlington Experiment Farm, United States Department of Agriculture, or in those of the department of plant pathology, Cornell University, none of which at the time contained any citrus other than that employed in the tests. Leaves were inoculated in all cases. The method of inoculation was essentially that described by Winston (25). Small stromatic masses of the fungus, developed in culture, were placed on bits of wet cotton so applied that the fungus came in contact with the leaf surface, and the whole was then wrapped in waxed paper. After about 48 hours all of the coverings were removed. The checks were treated in the same manner except that no inoculum was used. From two to seven individual inoculations, with as many checks, were made for each kind of plant. On Cuban shaddock, inoculations were also made with culture 71, as representative of the other distinct but less usual type of cultural growth. The final readings on infection were made from 18 to 21 days after making the inoculations.

<sup>7</sup> The serial numbers cited are those of crop physiology and breeding investigations, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, by which the plants to be inoculated were contributed.

With the exception of the citrangequat, infection resulted in all the inoculated plants, although not on every leaf inoculated. No reisolations were made. The checks remained uninfected. Of seven leaves of Cuban shaddock inoculated with culture 71, four became infected; and of seven leaves of the same variety inoculated with culture 8, at least one became infected. In addition to establishing the pathogenicity of these two strains of *Sphaceloma fawcettii* on certain rutaceous hosts, these inoculations serve to show that an isolation grown continuously in pure culture for as many as 11 years is still capable of producing infection. They also furnish a record of the fact that two isolations from different sources, markedly different in their gross cultural characteristics, were not physiologically different in their ability to infect a common citrus host, Cuban shaddock, on which no infection had previously been observed (26).

#### SUMMARY

This paper presents data pertaining to the development of conidia and conidiophores of *Sphaceloma fawcettii*, the causal fungus of citrus scab in Florida, especially as based upon the superficial development of the fungus in Florida on spring and autumn growth of leaves of sour orange, grapefruit, Tahiti lime, and young fruit of grapefruit. Other data included deal with cultural studies, inoculation tests, and secondary fungi associated with this fungus. Mention is made of the occurrence of *S. fawcettii* in Florida as early as 1878 and of probably the same fungus in South America in 1882 as well as its distribution in Java. The inoculation tests proved the pathogenicity of the organism on citrus after it had been grown in culture for a period of 11 years, and also its power to infect Cuban shaddock. In connection with the cultural studies of the fungus, colonies of two markedly different types are reported.

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# THE MECHANISM OF SEX IN UROMYCES APPENDICULATUS AND U. VIGNAE<sup>1</sup>

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## INTRODUCTION

The statement has been often reiterated that the spermogonia of the Uredineae are functionless male organs, having at most a doubtful physiologic value. Since the work of Blackman (7)<sup>3</sup>, Blackman and Fraser (8), and Christman (10, 11), and later confirmation by Olive (32, 33), Dodge (20, 21), Fromme (22, 23), and others, it has generally been held established that fertilization in the rust fungi consists in a fusion of equal gametes in the aecial primordium which results in the formation of a binucleate basal cell from which the chain of binucleate aeciospores and intercalary cells is produced. The fact that many investigators previous to Blackman and Christman, and a few since, have failed to observe the occurrence of such fusions shows the difficulty with which they may be observed and also their doubtful frequency. Later cultural work by Craigie (13, 14, 15) indicates that the spermatia must be assigned some part in the fertilization process.

In demonstrating the heterothallism of *Puccinia graminis* Pers. and a few other rust organisms, Craigie has shown that the nectar containing the spermatia is directly associated with maturation of spores in the aecium. He has shown that when nectar from a + spermogonium is applied to one portion of an undeveloped - pustule, the treated section of the pustule will form mature aecia while the untreated area remains sterile. Craigie's results have been confirmed in part by the writer, using the aecial stages of *Uromyces appendiculatus* (Pers.) Fries from beans (*Phaseolus vulgaris* L.) and *Uromyces vignae* Barclay from the cowpea (*Vigna sinensis* (L.) Endl.). The two forms have only recently been distinguished by Fromme (24) on the basis of a slight difference in spore characters. They are both full-cycle rusts, showing little if any physiologic specialization.

## METHODS

A method of inoculation was used in which the teliospores were germinated on water drops before they were applied to the host leaves. In this way the sporidia were separated and a maximum number of isolated sori obtained. By this method the first rust flecks appear on the third day after inoculation. Small droplets of nectar containing spermatia appear about the sixth day on the upper leaf surface.

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<sup>2</sup> Acknowledgments are made to R. F. Griggs, professor of botany at the George Washington University, and to W. W. Diehl, lecturer in mycology at the George Washington University and associate pathologist in the Bureau of Plant Industry, for their helpful interest and suggestions during these investigations, and to L. L. Harter, senior pathologist in the Bureau of Plant Industry through whose kindness it has been possible to do the work within the bean-disease project and whose criticism has been helpful in the preparation of this paper. The writer is further indebted to B. O. Dodge, pathologist of the New York Botanical Garden, for careful reading of the manuscript and suggestions for its improvement.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 535.

In case fertilization takes place, aecia follow on the lower surface about the tenth day. No careful attempt was made to eliminate insects entirely, but it was conclusively shown that, where nectar was transferred by hand, the aecia became conspicuously swollen by the third day after transfer, whereas maturation was delayed several days or failed to take place where no such transfer was made. Material for cytological examination was killed and fixed in Carnoy's and in Flemming's weaker solution and embedded in commercial paraffin. The best results were obtained with material cut as thin as it could be conveniently sectioned,  $4\mu$  to  $5\mu$ —never more than  $7\mu$ . In thick sections the maze of cells is entirely confusing and the staining is less satisfactory. The triple combination of safranin, gentian violet, and orange G, prepared according to Colley's methods (12), was used throughout for staining. In a few cases erythrosin was used in combination with the triple stain in order to bring out the vegetative nuclei of the fungus.

An examination of sectioned material showed the presence of binucleate cells in the primordium within 24 hours after transfer of spermatia. On the third day after transfer the aecia contained extended chains of aeciospores. Material of the same age, but where nectar had not been transferred, showed no binucleate cells, except in rare instances where insect transmission of spermatia may have taken place.

#### ARRANGEMENT OF SEXUAL ORGANS

Figure 1, A, shows the usual arrangement of sori in the gametophytic stage of *Uromyces appendiculatus* on bean plants. In bean rust the aecial primordia form in a ring in the mesophyll tissue of the host leaf, and the spermogonia form a cluster of sori opening on the upper leaf surface. The arrangement is similar in the cowpea rust, but the aecial primordia do not form so definite a ring. A few spermogonia frequently occur on the lower leaf surface and a few aecia on the upper. Pustules on leaf veins or petioles usually show a pronounced linear progression.

The first macroscopic evidence of fertilization in the aecia is a swelling of the pustule on the lower surface of the leaf. The spermogonia apparently cease functioning immediately on fertilization of the aecia. This seems to be true whether the fertilization takes place at an early stage or is long delayed. In case of delayed fertilization the spermogonia continue to produce nectar until the host tissue is exhausted. In one such case of delayed fertilization a secondary ring of exudate was found surrounding the primary cluster. This pustule was not examined microscopically, but if there was present a secondary growth of spermogonia it indicates a remarkable adaptiveness for attracting insects and securing fertilization. Sections of material on which nectar had been transferred by hand show a film of spermatia in a gelatinous matrix well distributed over the epidermis at the infected area.

This obvious association of the spermatia with fertilization in the aecium encouraged a detailed cytological study of the process. The presence of hyphae conspicuously projecting through the stomata of the infected area and in close proximity to the spermatia-containing nectar was the first fact in a chain of evidence which has led the writer to conclude that there is present in these species of rust a sexual mechanism very similar to that found in the red algae.

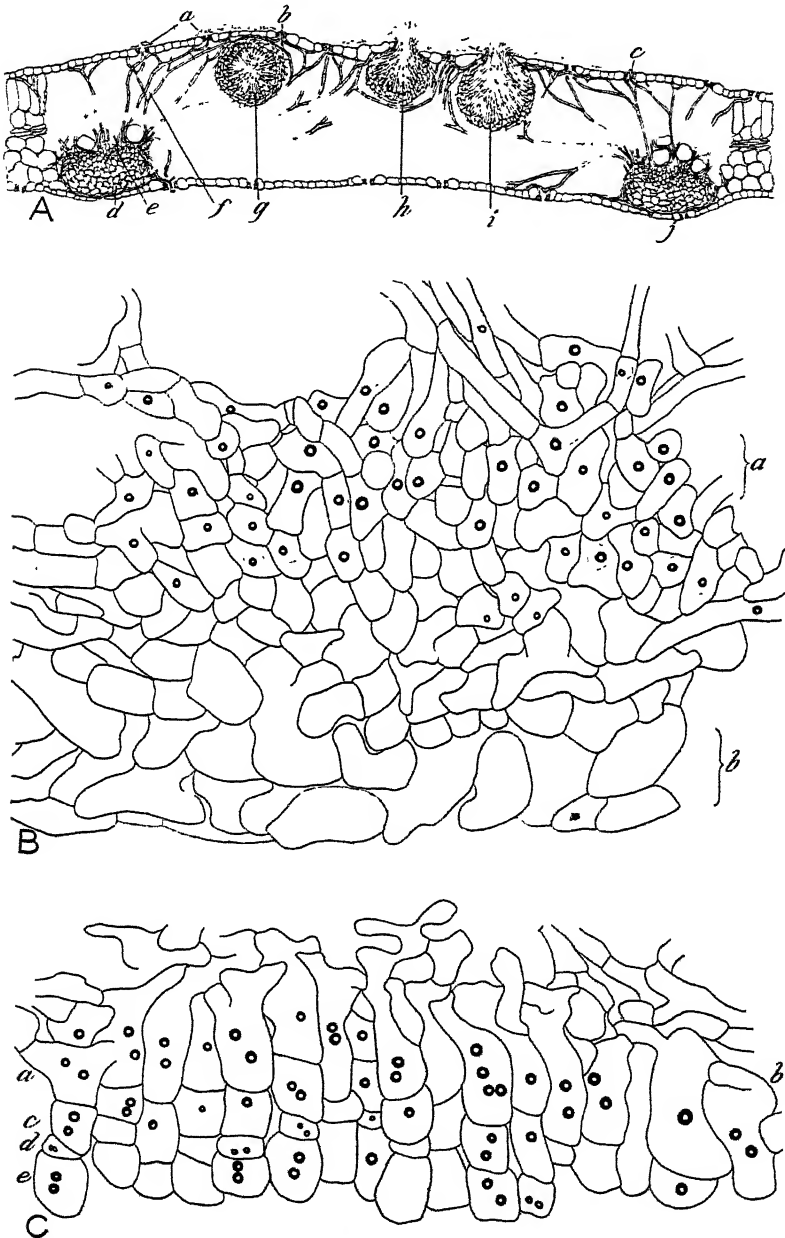


FIGURE 1.—Sexual mechanism in *Uromyces appendiculatus*.<sup>4</sup> A.—Semidiagrammatic drawing of infected leaf area showing relation of male and female elements at the time when fertilization is taking place: a, Film of nectar containing spermatia; b, tip of gametophytic hypha, ruptured at c; d, j, aecial primordia; g-i, spermatogonia; egg cells at e, with branched trichogynes, f.  $\times 73$ . B.—Perplexing arrangement of cells in the aecial primordium, showing egg cells (a) and pseudoparenchyma (b).  $\times 685$ . C.—Normal appearance of aecium at an early stage of maturity, showing fertilized egg cells (a, b), spore initial cell (c), intercalary cell (d), and young aeciospore (e).  $\times 685$

<sup>4</sup> In this and the succeeding legends the structures believed to function as egg cells, trichogynes, male nuclei, etc., are thus designated for purposes of brevity and clarity. The terms "fertile cell," "basal cell," and "egg cell" are used interchangeably.



## AECIAL PRIMORDIA

A close study was made to determine the structure and orientation of cells in the aecial primordia. These have been described only as tangled masses of hyphae in the early state, with later a differentiation of fertile gametes at the base and sterile buffer cells toward the epidermis of the host leaf. No mention had been found of any definite pattern of growth of these hyphae or of any definite habit of branching until a recent report by Allen (2). On casual observation the primordium appears to be no more than as described, but with the use of thin sections ( $4\mu$ – $5\mu$ ) definite structures can be traced. These are as shown in Figure 2, B to I. Extended observations have established the fact that this is a general condition in the forms studied. Figure 1, B, shows the baffling appearance of the primordium, but careful focusing reveals within this mass the presence of a larger number of 2-legged uninucleate cells with a constant habit of septation. (Fig. 2, D to G.) Owing to their peculiar orientation (fig. 2, C, G), never more than a few of these branched cells can be seen clearly in the same plane. In the earliest stage in the development of the primordium these cells are set at all angles, but later those nearest the epidermis of the leaf become disorganized and in part compose the pseudoparenchyma. Later, uninucleate cells are formed from the basal cells below and are added to the pseudoparenchyma. (Fig. 3, G, H, K, L, M.) At the point where fertilization would normally occur, the fertile cells become swollen and are crowded together in such a manner as further to obscure the connections with hyphae below. After fertilization, the elongation and continued expansion of the basal cells (fig. 3, I), spore mother cells, and aeciospores force a reorientation, so that the row of basal cells appears in nearly the same plane. (Fig. 1, C.) If fertilization does not take place at the maturation of the first fertile cells, development within the primordium may continue. The result is a chain of cells, each of which in turn could function as a fertile cell. From each cell of the series there extends a lateral, basal stalk, which might be interpreted as a trichogyne, as will be shown later. The terminal cells of the chain are vacuolate and lie within the pseudoparenchyma. Series of three fertile cells occur frequently, but none longer have been observed with certainty. This serial arrangement appears before fertilization, as shown in Figure 3, G, H, L, M, N. A further elaboration in structure may take the form shown in Figure 2, A. Such an extensive proliferation is infrequent in the uninucleate condition.

## COPULATORY ORGANS

The hyphae emerging through the epidermis provide an ample mechanism for fusion of spermatia with gametophytic mycelium. It was early discovered that the conspicuous organs in the open stomata (fig. 4, C) found in the first instance are not the most numerous and may not function as trichogynes. They probably represent an overdevelopment after failure to secure fertilization. They are most abundant in old primordia in which fertilization has not taken place, and are entirely absent in primordia fertilized as early as 10 days after inoculation.

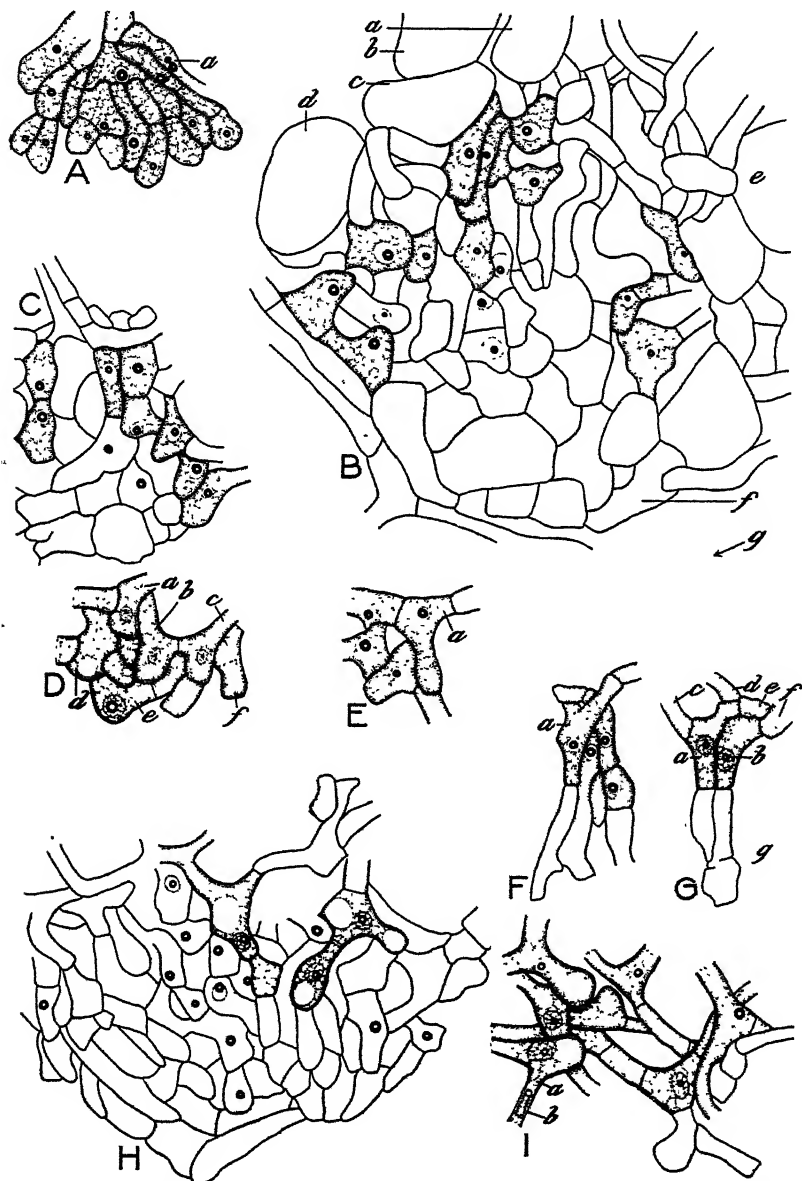


FIGURE 2.—Structure and arrangement of female cells in *Uromyces vignae*. A.—An exceptional case of proliferation before fertilization. Note the binucleate branch (a).  $\times 685$ . B.—Early stage in grouping of egg cells. Those showing the branched structure are shaded. Host cells are shown (a-e). Empty cells at f will compose the pseudoparenchyma. Lower epidermis is indicated at g.  $\times 685$ . C.—Slightly later stage than B, showing orientation of branched fertile cells (shaded) at lateral edge of a primordium.  $\times 685$ . D.—Section from base of a primordium, showing structural relation of female organs; fertile cells a to f.  $\times 685$ . E.—Group of egg cells showing habit of septation. The longer branch (a) usually functions as the trichogyne.  $\times 685$ . F.—Cells from primordium, showing manner in which the branching of the egg cell (a) is often concealed.  $\times 685$ . G.—Two fertile cells (a, b) with foot cells (c, d) and trichogynes (e, f). Empty cells extending into the sterile tissue at g.  $\times 685$ . H.—A young primordium, showing elaboration in structure of female organs.  $\times 685$ . I.—Section through lateral edge of a primordium, showing orientation of cells. Trichogyne (a) with male nucleus (b) just entering the fertile cell.  $\times 685$ .

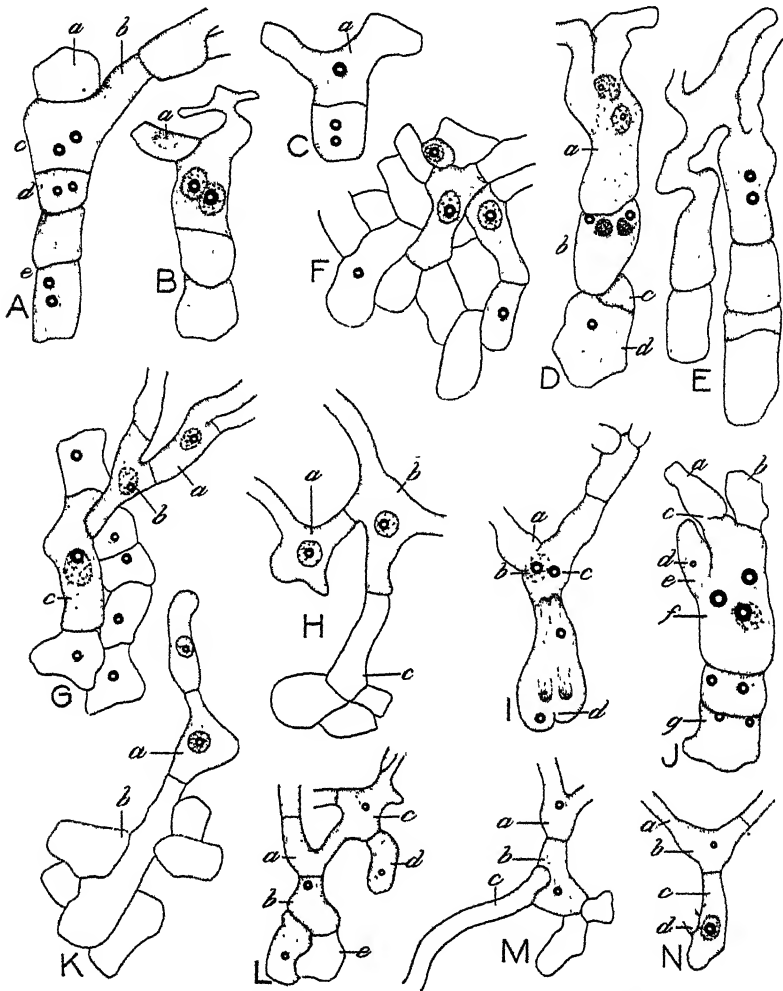


FIGURE 3.—Structure of female cells before and after fertilization in *Uromyces appendiculatus* (A–G, I, J, N) and *U. vignae* (H, K, L, M). A.—Fertilized basal cell (c) with stalk cell (a) and trichogyne (b). Spore initial cell (d) with portion of aeciospore chain at e.  $\times 830$ . B.—Similar to A, showing faint, disintegrating nucleus in the branch at a.  $\times 830$ . C.—Recently fertilized egg cell. Area at a shows position of second nucleus. Note similarity to F and N below. Stalk cell and trichogyne can not be distinguished.  $\times 830$ . D.—Same material as C, showing branched basal cell (a), spore initial cell (b) with paired nuclei preparing to divide, and intercalary cell (c), with young aeciospore (d).  $\times 830$ . E.—Linked basal cells after each has begun to produce a chain of spores. Septations have been dissolved.  $\times 830$ . F.—Typical appearance before fertilization of branched basal cells shown in A, B, C, and D.  $\times 830$ . G.—Linkage between basal cells before fertilization. After a and b had each begun to produce a spore chain they would probably appear as in E, above. What is possibly a third fertile cell of the series is shown at c.  $\times 830$ . H.—Another type of linkage between egg cells (a, b) previous to fertilization, showing empty cells (c) extending into the pseudoparenchyma.  $\times 830$ . I.—Branched egg cell soon after fertilization, showing that a is probably not a fusion cell. (Cf. fig. 8, B.) The nuclei, b, c, were distinct in stain and structure. What is probably the beginning of proliferation appears at d.  $\times 830$ . J.—Same material as I, showing fertilized basal cell (f), with trichogyne (a), stalk cell (b), and lateral fusion cell (e) with a small, faint nucleus (d). Note the break in the septum at c. The adjacent section shows a third nucleus in cell g.  $\times 830$ . K.—Early appearance of egg cell (a), showing similarity to Allen's "irregular swelling." Portion of sterile tissue at b. Compare with L, M, and N.  $\times 830$ . L.—Linkage between egg cells (a, c, and probably b and d). Sterile cells at e. Compare with H.  $\times 685$ . M.—Linkage between egg cells (a, b). The trichogyne (c) extends into the pseudoparenchyma. Compare with N.  $\times 685$ . N.—Egg cells (b, c) with trichogynes (a, d). This serial growth of fertile cells accompanies delayed fertilization. Compare with C. This indicates also a possible interpretation of J, in which e of J would be a second trichogyne, corresponding to d of this figure.  $\times 685$

But in addition to these conspicuous organs there are other ways in which the gametophytic mycelium may make contact with spermatia distributed on the surface of the host leaf. There are numerous cases in which the spermatia have entered through the open stomata and lie in contact with the mycelium. Figure 5, B, shows such a case. This is situated directly below an aecium that has just begun to produce binucleate cells. Hyphae lying parallel to the epidermis (fig. 5, C, D) are frequently seen to follow the periphery of the primordium or aecium and are lost in the tangle of mycelium at the base of the aecium.

The stomatal inclusions of the type shown in Figure 4, A, are still more numerous. These have no special structure but are often much constricted in passing between the guard cells and are swollen at the extremity. In material sectioned previous to fertilization these hyphal tips take a deep violet stain. In sections of material containing fertilized aecia the tips are frequently found shriveled and disorganized, showing only the orange of the triple stain. (Fig. 4, I, K.) In aged, sterile material—that is, material of unfertilized aecia three weeks or more after inoculation—there are found tips of hyphae projecting out between the guard cells, which are deeply stained with safranin. (Fig. 5, E.) This staining reaction is characteristic of disintegrating tissues or organs which have passed the functioning stage and become senescent (4).

In certain material numerous hyphae extrude directly between the epidermal cells. (Fig. 6, A to F.) As soon as these structures were found much of the earlier material was reexamined and many such cases discovered, all of which had previously been overlooked. They are either absent or not at all apparent in some material. They are most general on host material where the constrictions between epidermal cells are especially pronounced. In such material they may be so numerous as to provide alone a sufficient mechanism for copulation.

These facts concerning the occurrence of gametophytic hyphae protruding through the epidermis of the host leaf are by themselves not particularly suggestive. Such a thing might be expected of any internal parasitic fungus. But it is important to mention that such erumpent hyphae are not found in the telial stage of bean rust. The possibility was borne in mind that these isolated hyphae projecting beyond the epidermis might be nothing more than paraphyses or sporophores of undeveloped or reduced spermogonia. As a matter of fact what have been interpreted as much reduced spermogonia do occur rarely. One of these is represented in Figure 5, A. Except for a considerable enlargement, these appear to be structurally identical with spermatophores present in a mature spermogonium. They are certainly homologous. From their staining reaction and nuclear condition it is possible that they do not have the same function as the hyphae described above. It should be emphasized, however, at this point that all these structures are borne on the same thallus, and the habit of septation and branching, as shown in Figure 6, H, is constant throughout all organs of the parasite. All such special structures may be said to arise from separate internodes of the same thallus and are in a sense homologous.

Other facts concerning the time and condition of the appearance of these stomatal hyphae and their association with other parts of

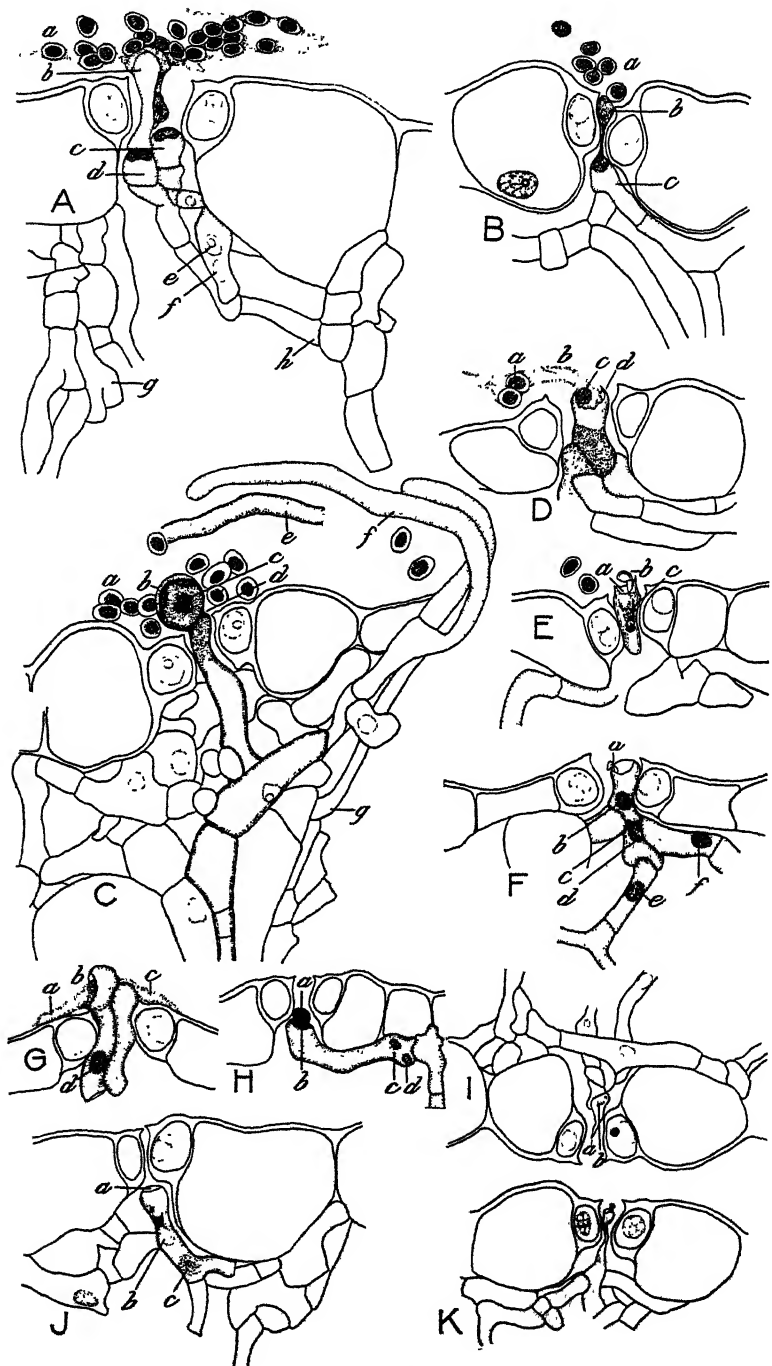


FIGURE 4.—Copulatory organs of *Uromyces appendiculatus* (A-E, H-K) and *U. vignae* (F, G)  
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the sexual mechanism, added to the mere fact of their occurrence, indicate that these hyphae are the tips of functioning trichogynes. At an early stage previous to fertilization, when the aecial primordia are in process of formation, that is, 8 to 12 days after inoculation, no hyphae are found in the stomata or protruding between epidermal cells. Instead, numerous elongated hyphal branches are found with their growing tips directed toward the epidermis and often just entering the substomatal cavity or deflected in the constrictions between epidermal cells. (Fig. 1, A.) The appearance of these strands suggests that they are growing toward the region on the epidermis where spermatia are distributed. In this early stage the primordium is a somewhat loosely formed structure. (Fig. 2, B.) At this time outgrowths from the fertile cells which could be interpreted as developing trichogynes are frequently found. (Fig. 2, H, and fig. 3, K, L.) Nectar transfer at this stage results in the fertilization of a few basal cells in some, but not all, of the primordia. The numerous chains of aeciospores which result arise from a proliferation of the few fertilized basal cells. (Fig. 7, D.) In such material cell fusions of various types are common. In exceptional cases there appears to be a rather general dissolution of cell walls. These fusion may or may not be accompanied by migration of nuclei. Many of these fused cells have the identical appearance of fusion cells described by other observers (10, 22) and interpreted by them to be "sex fusions."

At a later stage the hyphal tips are found projecting between the guard cells of open stomata or between epidermal cells. They are always conspicuously more numerous in the vicinity of spermogonia, but appear on both the upper and lower epidermis. No attempt has been made to determine the possible effect of humidity and light on the development of erumpent hyphae. It is possible that under conditions where stomata would normally be wide open the spermatia would enter freely so that any considerable extrusion of a copulatory organ would be unnecessary. (Fig. 5, C.) As a matter of fact in one lot of material, where the plants were kept in moist chambers for 48 hours following transfer of nectar, the sectioned material showed an almost entire absence of hyphae protruding beyond the plane of the guard cells. Spermatia, however, were frequently found within the

#### EXPLANATORY LEGEND FOR FIGURE 4

- A.—Partially sterile infection 36 days after inoculation. Stomatal hyphae (b-d) associated with mass of nectar and spermatia (a). The hypha (b) appears to have a gelatinous sheath. Note the deeply stained receptive spots and the swollen tips of c and d. Faint nuclei (e, f) and gametophytic hyphae (g, h).  $\times 830$ . B.—A possible germinated spermatium (b), but more probably a prolongation of the terminal cell of the trichogyne (c). Spermatia at a.  $\times 830$ . C.—Abnormal development of copulatory organ: a, Spermatia; b, gelatinous sheath. The nuclei at c and septation at d are both uncertain. Paraphyses (f) projecting from ostiole of spermogonium (g), and section of broken paraphysis (e).  $\times 830$ . D.—Terminal cell of trichogyne, showing ruptured tip (d) with the male nucleus (c) just entering. In size and staining reaction c is identical with the nuclear contents of spermatia at a. Nectar at b. Compare with F and G.  $\times 830$ . E.—Severed tip of trichogyne (a), with male nucleus present as an indefinite mass at c. The discarded shell of the spermatium is seen on the lip of the ruptured hypha (b). Compare with F.  $\times 830$ . F.—Tip of trichogyne with portion of spore case caught on the lip of the ruptured hypha (a). The male nucleus (b) is scarcely distinct from cell nuclei (d-f). The septation at c was not clear.  $\times 685$ . G.—Terminal cells of two gametophytic hyphae. One hypha ruptured at b. Nectar is seen at c. The exudate (a) may be partly composed of cytoplasm from the ruptured hypha. The male nucleus may be present at d.  $\times 685$ . H.—Terminal cell of a gametophytic hypha with a spermatium resting in a slight depression at the tip (a). The receptive spot (b) is stained violet. Two indistinct bodies (c, d) may be nuclei.  $\times 685$ . I.—Shriveled tip of disintegrating trichogyne (a) from material with mature aecia. Nuclear material at b is probably remnant of cell nucleus.  $\times 685$ . J.—Substomatal hypha with ruptured tip (a). Two indefinite nuclei are present (b, c). The former is probably the male nucleus and the latter the usually indistinct cell nucleus. Compare with Figure 6, G.  $\times 685$ . K.—Ruptured and shriveled tip of a trichogyne from material with mature aecia. Such disintegration normally follows passage of the male nucleus. Compare with J.  $\times 685$

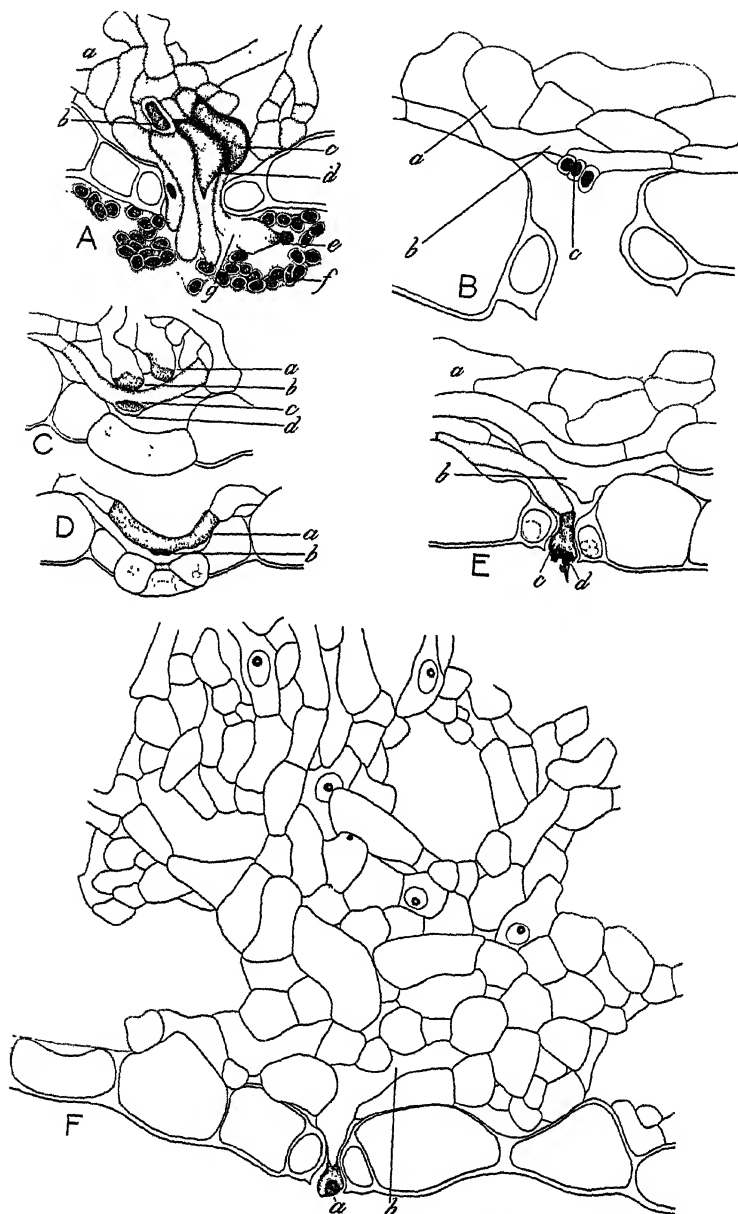


FIGURE 5.—Stomatal structures of *Uromyces appendiculatus*  
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open stomata and in contact with the hyphal tips, occasionally appearing to be in process of fusion with the hyphae. On the other hand, material from cowpea leaves cultured in Petri dishes showed high development of erumpent hyphae. The most elaborate development of these organs is found in unfertilized material killed 17 days or more after inoculation. As many as three have been seen protruding through the same stoma. In a number of cases erumpent hyphae have been found growing along the surface of the leaf for some distance. (Fig. 6, C.) The hyphal tips normally take a deep stain, usually violet in the earlier stages and safranin in the later stages. Where the whole terminal cell is not stained heavily, there is in almost every case a darkly stained area at the extremity which at times has the appearance of nuclear material, but more probably represents a receptive spot, possibly such as is found on the unfertilized female cells of other organisms, for example, *Vaucheria*, and the eggs of various vertebrates.

A study of the internal mycelium reveals a peculiar method of branching by which means the gametophytic hypha progresses along the host epidermis and emerges at any convenient outlet which might be by chance in the region of spermatia. (Fig. 1, A.) Staining reactions indicate that as later branches are formed the earlier ones become vacuolate. The strands usually incline upward and toward the spermogonia opening on the surface of the leaf at the center of the pustule. They may, however, form a parallel web of mycelium beneath the epidermis, extending for a considerable distance away from the center of the infected area. These subepidermal strands may become very numerous where fertilization has not taken place promptly. The strands are highly septate and the cells uninucleate. The internodes are of irregular length and at times quite prolonged. (Fig. 6, H.) In certain material the presence of a second nucleus is distinguished in individual cells. (Fig. 6, I.) The nuclei stain very poorly and are usually seen with difficulty. They are often considerably elongated and in some cases appear to be partially disorganized. Some possess small nucleoli, others do not. After maturation of the aecia, most of the surrounding hyphae assume an empty and lifeless appearance. In old sterile material the hyphae undergo numerous and peculiar forms of branching and anastomosing.

#### DISCUSSION OF SEXUAL MECHANISM

Assuming that fertilization does take place by means of a trichogenous hypha extending from the base of an egg cell and terminating at the surface of the host leaf, one must conclude, first, that fusions of cells of parallel hyphae, with accompanying migration of nuclei, when they do occur, can not constitute fertilization. In view of the

#### EXPLANATORY LEGEND FOR FIGURE 5

A.—Group of hyphae representing what may be a much reduced spermogonium: *a*. Gametophytic mycelium, *b-d*, deeply stained areas; *f*, mass of nectar and spermatia. A possible fusion of hypha tip with a spermatium is at *e*, and a break in the hypha at *g*.  $\times 830$ . B.—Open stoma beneath an aecium, showing spermatia (*c*) lying in contact with mycelium (*b*) at border of pseudoparenchyma (*a*). Compare with F.  $\times 830$ . C.—Gametophytic hyphae underlying a stoma, showing a rupture at *c*. The regions *a*, *b*, and *d* are brightly stained with orange G.  $\times 685$ . D.—Similar to C. The cell *a* is stained dark violet, while the region at *b* is still more deeply stained.  $\times 685$ . E.—Old and partially sterile infection with pseudoparenchyma shown at *a* and mycelium at *b*. The lifeless terminal cell (*c*) has taken a deep safranin stain. The formless material (*d*) may be part of the ruptured hypha tip.  $\times 830$ . F.—An aecial primordium showing a hypha projecting through an open stoma with a possible fusion with a spermatium at *a*. The cell *b* is apparently a part of the pseudoparenchyma. Branched egg cells are shown with nuclei.  $\times 830$



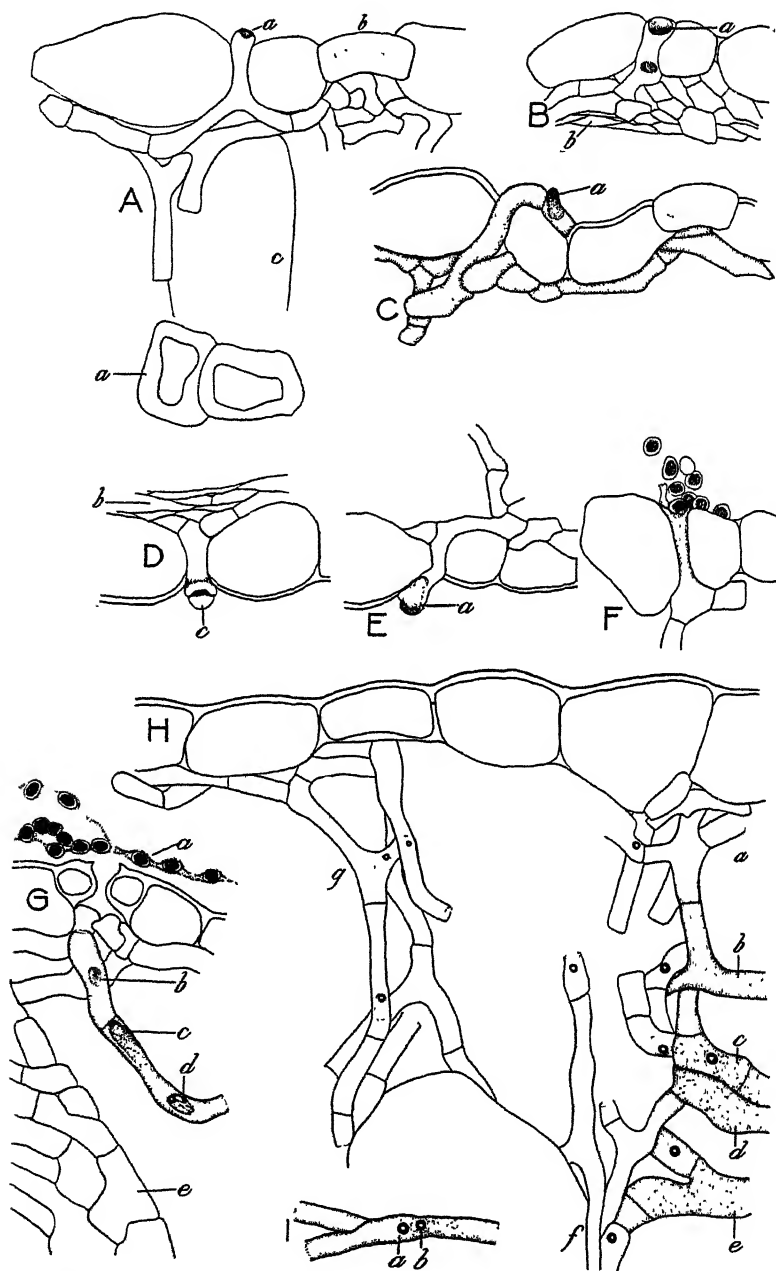


FIGURE 6.—Copulatory mechanism in *Uromyces appendiculatus*. (F is from *U. vignae*)

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many published accounts there can be no question that parallel cell fusions do occur in a considerable number of rust forms. However, the writer has recently examined slides of *Kunkelia nitens* (Schw.) Arth. and is prepared to question many of the conclusions drawn from the observations made on this and similar forms. While structures resembling fusion cells are very numerous on certain of these slides, in many cases at least the manner of junction of the cells is such that they can not, from the standpoint of morphology alone, be interpreted as fusion cells. The evidence indicates that cell fusions occur in the two forms included in the present study, but occur with frequency only when conditions are unfavorable for best development and nonconducive to fertilization in the parasite. The few clear cases of a nucleus in actual process of migration between cells of parallel hyphae show a nucleus migrating into a cell which is already binucleate. (Fig. 8, A.) Parallel cell fusions passed unnoted by investigators previous to the work of Blackman and Christman; since then opinions have been divided as to their actual occurrence and significance. Yet for the most part Christman's conclusions have been accepted. The extreme height of conviction is amply demonstrated in the report by Fromme on sexual fusions in the flax rust, *Melampsora lini* (Pers.) Desmaz. (22). On the other hand, Allen (2) and Kursanov (29) state definitely that they find no indication of fusion in their material of *Puccinia graminis*, and so make advisable a complete reexamination of rust forms in which cell fusions are reported as constituting fertilization.

In the second place, it must be concluded, on the above assumption, that the row of binucleate basal cells present in the mature aecium, and stated to be 2-legged fusion cells by previous observers, are not necessarily fusion cells but are structures which may be present in the primordium previous to fertilization, that is, in the uninucleate condition. This is clearly the case, as a comparison of Figure 3, A to E, with Figure 3, F, G, and Figure 8, B, will show. It is readily seen that uninucleate branched cells present in the primordium might easily be interpreted as 2-legged fusion cells when in the binucleate condition. It is true that these basal cells form vegetative fusions with parallel hyphae in particular cases. In Figure 3, J, the two branches of the original uninucleate basal cell are shown in addition to the lateral fusion cell. However, the possibility should not be overlooked that this lateral cell (*d*) may represent a second trichogenous hypha. Perhaps normally a cross wall would have divided

#### EXPLANATORY LEGEND FOR FIGURE 6

A.—Hypha projecting between epidermal cells, showing deeply stained receptive spot (*a*); stoma at *b*, and host palisade cell at *c*.  $\times 685$ . B.—Hypha projecting between epidermal cells beneath a mature aecium, showing ruptured tip (*a*), and pseudoparenchyma (*b*). Note presence of nucleus in the terminal cell, as contrasted with A.  $\times 685$ . C.—Gametophytic hypha growing on surface of host leaf. The nucleus (*a*) may represent a spermatium in process of fusion with the hypha.  $\times 685$ . D.—Hypha projecting between epidermal cells beneath a mature aecium, showing peridial cells (*a*) with crushed cells of the pseudoparenchyma (*b*). The empty violet-stained structure (*c*) is probably not a spermatium.  $\times 685$ . E.—Probable fusion of a male nucleus with tip of trichogyne protruding between epidermal cells (*a*). Compare with C.  $\times 685$ . F.—Ruptured tip of hypha of *U. vignae* appressed between epidermal cells, showing close association of spermatia.  $\times 685$ . G.—Gametophytic hyphae at a stoma near ostiole of a spermatogonium, showing migrating male nucleus in trichogyne (*c*), and cell nuclei, disintegrating (*b*) and still functional (*d*). The terminal cell of this hypha has probably been sectioned away. Nectar with spermatia is shown at *a*, and border of spermatogonium at *e*.  $\times 685$ . H.—Section from lateral edge of an aecial primordium showing branching habit of hyphae. Fertile cells (*b-e*) are seen at the edge of the primordium, with branches extending out to the epidermis (*a, f*); indicating also the aggregation of hyphae beneath stomata (*g*).  $\times 685$ . I.—Section of a hypha from beneath an aecium where fertilization is in process. The two nuclei (*a, b*), represented here only by the nucleoli, are distinguished by their staining reaction. The violet-stained nucleus (*b*) is probably the male nucleus.  $\times 685$

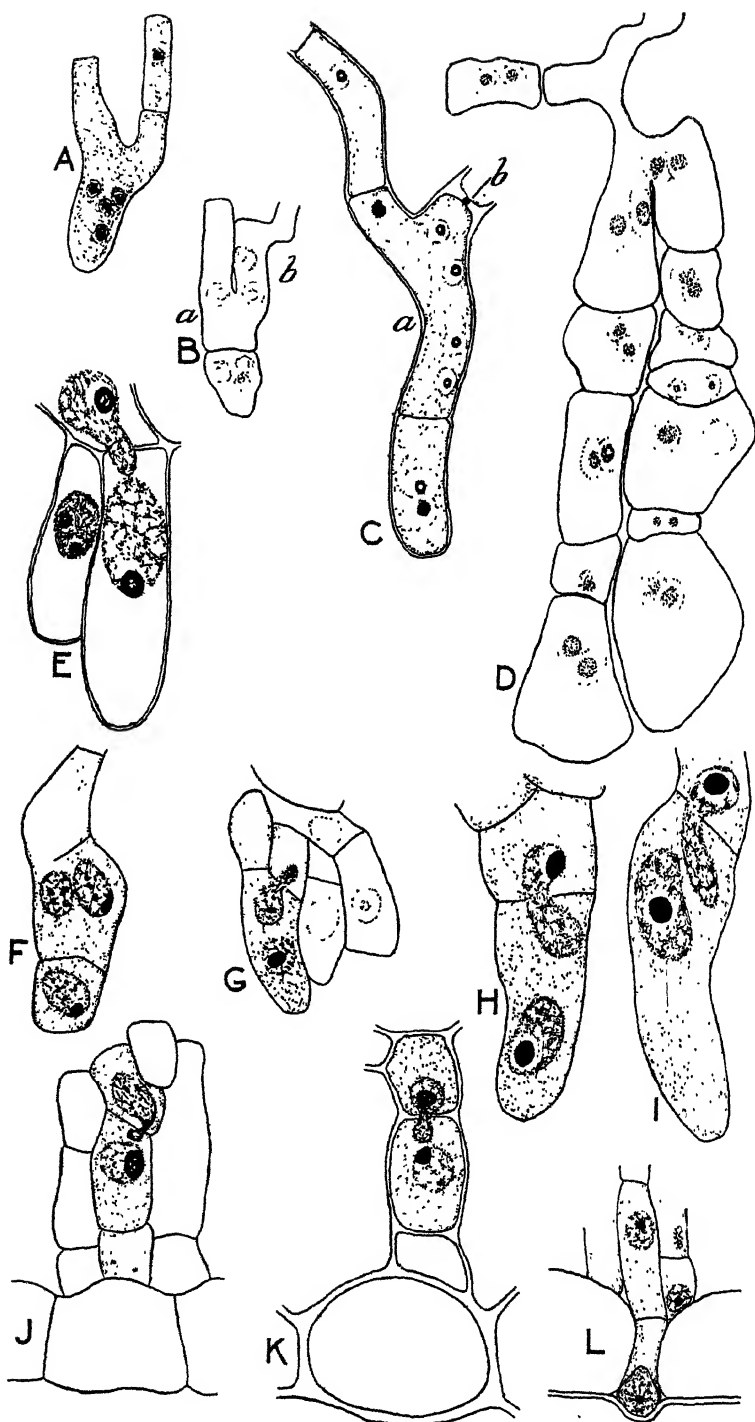


FIGURE 7.—Gametophytic structures in other Uredineae  
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the cell (f), as has occurred in Figure 3, N. This is drawn from material 17 days after inoculation in which no nectar was transferred. Only a few aecia show binucleate cells. The presence of three nuclei in the basal cell should be noted, as well as the faint nucleus in the lateral cell (d). The break in the septum of one foot cell is also suggestive.

In the third place, there should be present, in the primordium, egg cells, each with a branch representing the trichogyne and probably a second stalk representing the foot cell. These structures are present, as described above. After fertilization and subsequent expansion and reorientation, they are represented in the two forms studied by the row of 2-legged basal cells in the aecium (fig. 1, C), similar to what has heretofore been commonly figured.

With a carpogonic structure present such as suggested above, the binucleate condition should be found to arise from the migration of a nucleus from a cell below and continuous with the egg cell rather than from an adjacent cell. While migrations of this sort are not easily seen, they are quite numerous and more regular in occurrence than are migrations between cells of parallel hyphae. Several factors may account for the extreme difficulty in finding the male nucleus in actual process of entrance into the egg cell. For one thing, fertilization of the large number of egg cells in the same aecium, while normally occurring in close succession, does not take place simultaneously. It is extremely unlikely, therefore, that more than a very few male nuclei would be seen in a significant stage of migration in the same aecium. Again it may be that the wall dividing the egg cell from the trichogenous hypha is not formed in some cases until after entrance of the male nucleus, in which case the break in the septum often found following nuclear migration would be absent. (Fig. 7, F, and fig. 8, F.) Migrations of the type described by Blackman (7) took place through a very small pore in the septum which was not visible either before or after passage of the nucleus. (Fig. 7, J, K.) Bachmann (4) reports a transitory opening in the cross walls of the trichogyne of *Collema*, while Harper (26) reports a similar transitory opening between the oogonium and the conjugation tube in *Pyronema*. Furthermore, the difficulty of tracing the continuation of egg cell and trichogyne in the compact aecium would prevent finding the whole mechanism in the proper plane except in rare instances.

#### EXPLANATORY LEGEND FOR FIGURE 7

[These illustrations have been redrawn and inverted]

- A.—*Puccinia caricis*. A supposed fusion cell. Compare with Figure 3, N.  $\times 750$ . (After Kursanov, 29.)  
 B.—*Cronartium ribicola*. Diagram of an irregular basal cell. A lateral fusion may have taken place at a, or the three nuclei may have resulted from the dissolution of a wall at b. Compare with Figure 8, C and D.  $\times 850$ . (After Colley, 12.)  
 C.—*Uromyces scutellatus*. Hyphal connections of basal cell (a) after fertilization. A supposed fusion cell, but compare with Figure 9, G to K. The point b may represent a pore in the septum.  $\times 1,500$ . (After Kursanov, 29.)  
 D.—*Endophyllum sempervivi*. Two spore chains arising from proliferation of a single basal cell. Magnification not given. (After Hoffmann, 27.)  
 E.—*Triphragmium ulmariae*. Nucleus migrating into the fertile cell from cell below.  $\times 1,500$ . (After Kursanov, 29.)  
 F.—*Phragmidium violaceum*. A binucleate cell, showing the broken septum and the empty appearance of the cell from which the second nucleus has migrated. Compare with Figure 8, F.  $\times 1,300$ . (After Welsford, 32.)  
 G.—*P. violaceum*. Migration of nucleus from one of the two basal branches of the fertile cell. Compare with Figure 8, G, and Figure 9, F.  $\times 1,300$ . (After Welsford, 32.)  
 H, I.—*Triphragmium ulmariae*. Nuclear migration between continuous cells.  $\times 2,350$ . (After Lindfors, 30.)  
 J.—*Phragmidium violaceum*. Early stage of nuclear migration.  $\times 1,350$ . (After Blackman, 7.)  
 K.—*P. violaceum*. Nuclear migration between cells of same hypha.  $\times 1,350$ . (After Blackman, 7.)  
 L.—*P. violaceum*. Sterile cell of aecial primordium grown up between epidermal cells and covered only by the host cuticle.  $\times 1,350$ . (After Blackman, 7.)

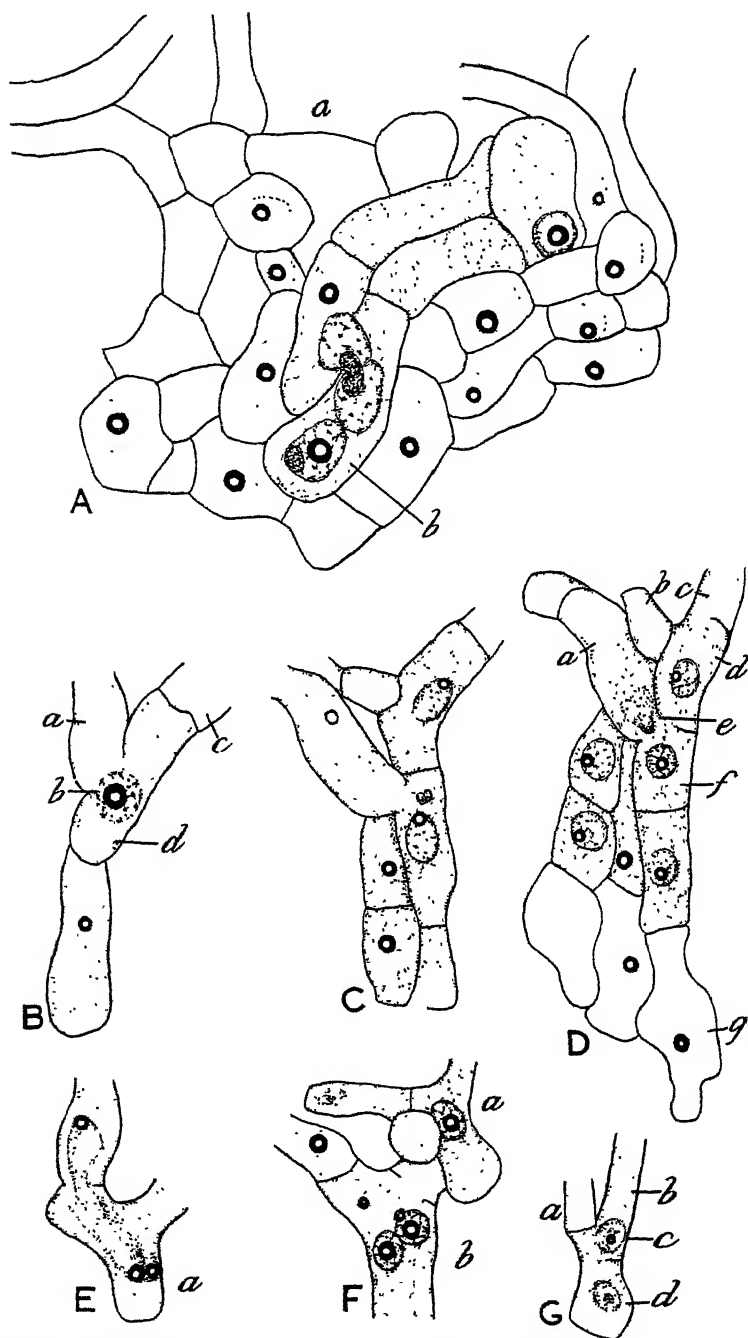


FIGURE 8.—Nuclear migration and fertilization in *Uromyces appendiculatus* (A, B, E, and G) and *U. vignae* (C, D, and F)  
For explanatory legend see opposite page

Examples of nuclear migration between continuous cells in bean and cowpea rust fungi are shown in Figure 8, C to G, and Figure 9, A to K. A suggestive fact is the frequent dissimilarity in size and structure of the paired nuclei before the first conjugate division. One is the large, well-defined nucleus with a very large nucleolus found in each of the fertile cells before fertilization; the other is small and poorly defined, often lacking a nucleolus but appearing rich in chromatin material. It should be recalled that the nucleus of the spermatium, while large as compared with the size of the spermatium as noted elsewhere, is a small deeply staining body without a nucleolus, while the vegetative nuclei below the fertile cells, although small, have a large nucleolus and stain no differently from the single nucleus in the fertile cells. Illustrations reproduced in Figure 7 show that migrations of nuclei into the fertile cell from a cell below and continuous with the fertile cell have not passed unobserved in other rust forms.

It would of course be desirable to trace the trichogenous branch extending from the egg cell through the host tissues and mass of vegetative mycelium of the fungus until it terminates in association with male cells. The difficulty of doing this by the usual serial-section method is obvious. In no case has it been possible to follow a particular hypha from an egg cell to its terminus at the surface of the host leaf. The most that has been accomplished is to trace hyphae from the egg cells to a point free from the aecium and to trace hyphae from their extremity in the stomata to a point where they are lost among the cells of the aecium.<sup>5</sup>

Finally, if copulation of spermatia with trichogynes takes place, these should be found in actual process of fusion. In fact many cases have been seen which strongly suggest that a fusion of spermatia with stomatal hyphae is taking place or has taken place. Figure 4 shows various stages in the fusion process. Hyphae showing ruptured tips are especially numerous. (Fig. 4, D, E, F, G, J.) Protected as they are between the host cells, there is no reason to suppose that these ruptures are entirely the result of accident. Numerous also in certain material are the shriveled tips represented in Figure 4, I and K. Spermatia are in close association with the unruptured tip in Figure 4, A. A spermatium in a depression at the tip of the hypha is shown in Figure 4, H. Figure 4, E and F, show what appears to be the discarded spore case of a spermatium caught on the lip of the ruptured hypha. Within the one hypha is found an irregular mass resembling nuclear material, while in the other a definite nucleus

<sup>5</sup> The interpretative nature of structures here designated as "egg cells" and "trichogynes" should be emphasized at this point. Some observers will probably maintain that these egg cells bear no more than a functional relationship to egg cells in algae or other organisms, and that the trichogenous branch is in fact no more than a simple vegetative hypha.

#### EXPLANATORY LEGEND FOR FIGURE 8

A.—Nuclear migration between cells of parallel hyphae. The cell *b* already contains two nuclei, suggesting a previous migration from another source. The base of the primordium is at *a*.  $\times 1,660$ . B.—Fertile cell (*d*) previous to migration of male nucleus, showing trichogyne (*a*) and stalk cell (*c*). Such a constriction (*b*) at the junction of egg and trichogyne is frequently found both before and after fertilization. Compare with C and D and with Figure 3, I.  $\times 1,660$ . C, D.—The same structures as they appear in adjacent sections. Two egg cells (*d, f*) in series, with trichogynes (*a, c*), stalk cell (*b*), and migrating male nucleus (*e*). A second nucleus in cell *d* is shown in C.  $\times 1,370$ . E.—Nuclear migration between continuous cells. Apparently two nuclei are already present in the egg cell (*a*).  $\times 1,370$ . F.—Two egg cells, before (*a*) and soon after fertilization (*b*).  $\times 1,370$ . G.—Male nucleus (*c*) migrating from the trichogyne (*b*) into the female cell (*d*). Stalk cell at *a*. Compare with Figure 7, F and G.  $\times 1,370$

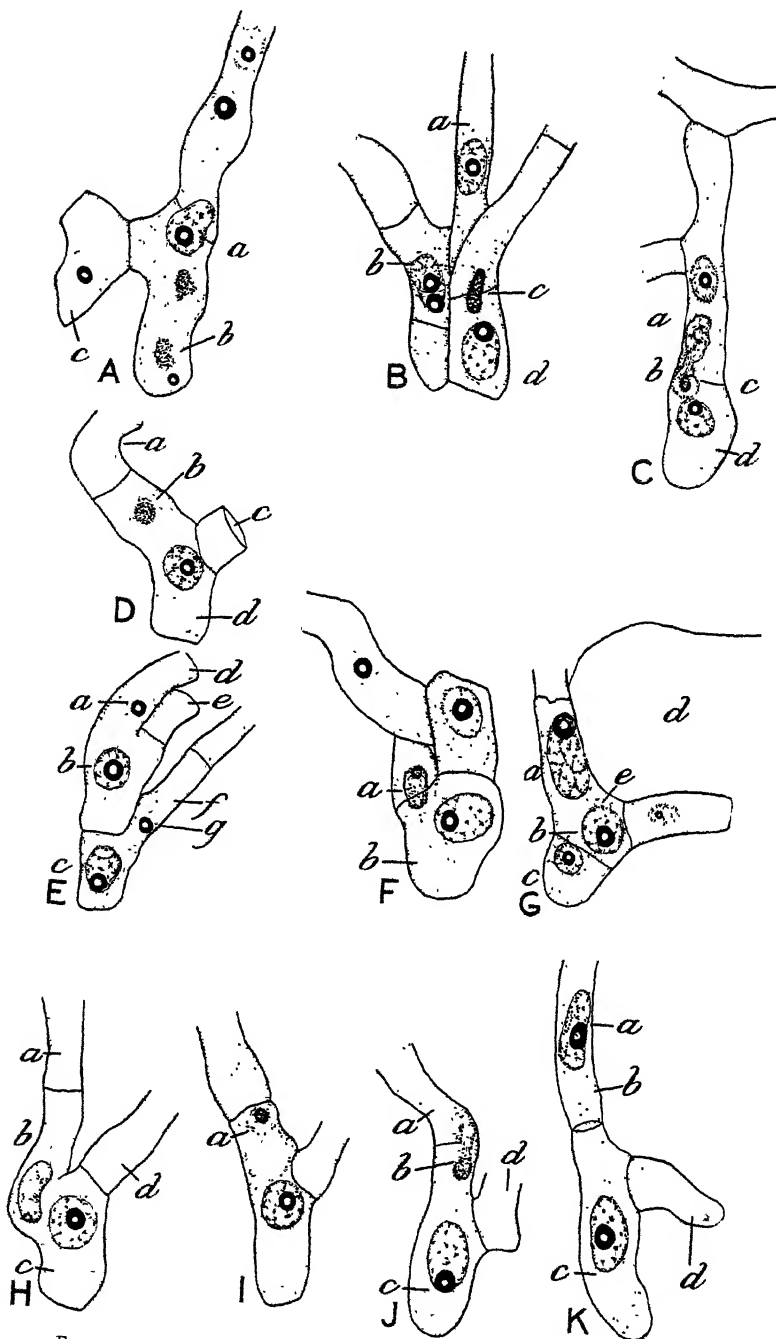


FIGURE 9.—Fertilization in *Uromyces rigiae* (A, C) and *U. appendiculatus* (B, D-K)  
For explanatory legend see opposite page

is visible, with a second very lightly stained body present. In few cases do the terminal cells of unruptured stomatal hyphae show a definite nucleus, while the cells below commonly have a single poorly stained but usually definite nucleus. The terminal cells of the ruptured hyphae occasionally show two nuclei, usually not well defined. Figure 4, J, shows such a case. The frequent occurrence of two nuclei of different appearance in the same hyphal cell strongly suggests the migration of a foreign nucleus. (Fig. 6, G.) In fact, nuclei have been seen passing through the septa between hyphal cells. A small pore can be seen in many of these cross walls, and in addition they are often found ruptured, supposedly after passage of the male nucleus.

#### RECAPITULATION AND REVIEW OF LITERATURE

In reviewing the literature on cytology and sexuality in the Uredineae, many of the above observations are found supported by the work of previous investigators. It is believed that the present interpretation will harmonize many conflicting opinions, and that many cases of exceptional behavior in the rusts will come to have a meaning. Several observers have suggested the relationship of the Uredineae to the Rhodophyceae. The uninucleate sterile cells budded off previous to fertilization were regarded as vestiges of once functional trichogynes. It is possible that it has not occurred to anyone to seek for vestiges of a trichogyne at the base of the fertile cell. No one has maintained that the fertile cell in the rust aecium is in a true sense a functional egg cell. Migrations of nuclei into the fertile cells from cells lying beneath and continuous with the fertile cells have been described for a number of species of rust. (Fig. 7.) But in most cases those reporting the occurrence of such migrations have maintained that migrations also take place between cells of parallel hyphae and that either type may constitute fertilization. Apparently, to most of them fertilization consists in a partial or complete fusion of equal gametes. Fromme (22) has even described the fusion of as many as four parallel cells to form a 4-legged basal cell. It would seem a point for controversy, then, not only when fertilization begins but when it ends.

The protrusion of gametophytic hyphae through host stomata is associated with at least several other rust forms, as revealed by a careful search of older publications. Recent observers have made scarcely any note of their occurrence, but in their drawings some have come very close to representing such structures. De Bary (5)

#### EXPLANATORY LEGEND FOR FIGURE 9

A.—Male nucleus (a) entering the fertile cell while the female nucleus is in process of division (b). Note similarity of c to b of Figure 2, D.  $\times 1,370$ . B.—Two egg cells, b fertilized, and d with male nucleus just entering at c. The hypha a is probably a second branch of d.  $\times 1,370$ . C.—Nuclear migration between continuous cells. Here a wall (c) has formed above the stalk cell (a). A male nucleus (b) is entering the egg cell (d). Compare with Figure 7, H.  $\times 1,370$ . D.—Basal cell (d) soon after fertilization, showing trichogyne (a), stalk cell (c), and male nucleus (b).  $\times 1,370$ . E.—Two egg cells in series and in same stage of fertilization, with male nuclei (a, g), female nuclei (b, c), trichogynes (d, f), and stalk cell (e). Compare with Figure 8, C.  $\times 1,370$ . F.—Male nucleus (a) entering female cell (b).  $\times 1,370$ . G.—A fertilized cell (e). Under some conditions the male nucleus (a) may continue through the wall at b so that the cell c would function as the basal cell of the spore chain. Host cell is shown at d. Compare with C.  $\times 1,370$ . H.—Basal cell (c) soon after entrance of male nucleus (b), showing trichogyne (a) and stalk cell (d).  $\times 1,370$ . I.—Basal cell just after fertilization, with male nucleus poorly outlined at a.  $\times 1,370$ . J.—Basal cell (c) with male nucleus entering at b, showing trichogyne (a), and stalk cell (d).  $\times 1,370$ . K.—Basal cell (c) just before entrance of the male nucleus (a), showing trichogyne (b) and stalk cell (d). H, I, J, and K show remarkably similar structures at different stages of fertilization.  $\blacktriangle \times 1,370$



in 1887 reported the frequent occurrence of stomatal hyphae. Richards (35, p. 257), describing the aecial stage of *Uromyces caladii* on Peltandra, says: "Occasional hyphae were seen protruding out of the stomata, but they did not connect with any of the primordia, and showed no evidence of any specialization." Klebahn (28) reports seeing wefts of hyphae in open stomata from several species of *Aecidium*. He finds them often swollen at the tip and frequently associated with spermatia, but he concludes that they have no sexual function. He even speaks of artificially spreading spermatia over the leaf area where aecial primordia were awaiting fertilization, and concludes that they do not influence the maturation of the aecia. No reference has been found to this phase of Klebahn's work in any publication. Kursanov (29), Blackman (7), and a few others have shown gametophytic hyphae associated closely with an aecium crowding apart the epidermal cells. Allen (2) shows one hypha which has protruded between epidermal cells and extended for some distance along the surface of the host leaf. Her interpretation of the structure indicates that it has no such function as a trichogyne. There might be mentioned parenthetically a situation found in *Polystigma rubrum* DC. The sexual condition in this ascomycete is of a doubtful nature similar to that in the rusts, and Blackman and Welsford (9) describe gametophytic hyphae projecting through the epidermis at a very suggestive stage. In this connection it is well to add that Moreau and Moreau, in a recent paper (31), deny the occurrence of a true fertilization in any of the Ascomycetes or lichens. According to these investigators, copulation by means of a trichogyne in any of these forms is a myth.

Blackman perhaps more than any other has appreciated the fact that the spermatia of the rusts are male cells (7). He regards them as now functionless. It appears from his statements that all that is lacking to make them functional is a mechanism for bringing the spermatia in contact with the female cells at the base of the aecium. He says: "That the spermatia, though perfectly functionless, should still be produced by the majority of forms and in such great abundance is certainly a very striking phenomenon." He also notes the similarity of rust spermogonia to those of *Collema*, in which the sexual nature of the spermatia has been shown by Stahl (37) and Bachmann (3). More recently Dodge also has emphasized the sexual nature of the spermatia of the Uredineae (19, p. 1055). He states: "There being no organ such as an egg apparatus in the rusts, the spermogonia, though they are so well developed in many species, can not carry out their primary male sexual function." That the rust spermatia are not perfectly functionless has been proved by confirmation of Craigie's work. The presence of structures functioning as an egg apparatus now appears probable.

As Blackman states, the structure of the spermatia alone indicates their nature as male cells. The fact that they may produce germ tubes does not necessarily disprove their sexual nature. According to Blackman (7) a similar vegetative potentiality occurs in the gametes of some algae. Allen (2) recently reports what she interprets as germinating spermatia of *Puccinia graminis* on the leaves of barberry. She even suggests that the germ tube may enter through the ostiole of the spermogonium. She does not understand why, if this takes place, it is not seen more frequently, but suggests that

the spermatia may grow at night, in which case she would not have seen them since her material was fixed by daylight. She describes spermatia, each with what she calls short germ tubes extending from both ends, and in some cases germ tubes from two cells appear to have fused.

Structures having a similar appearance are found in the material from which the present study was made. In some cases several spermatia may be seen in a chain connected by an orange-staining unwallled strand. These in the present case are interpreted as being nothing but strands of nectar, which often stain very tenaciously and may have the appearance of mycelial threads. (Fig. 6, G.) The spermatia themselves are frequently attenuated, and are often seen with tails of nectar which give the semblance of germ tubes. The extremely thin spore walls in such cases are sometimes hard to distinguish from the nectar. But what might be a true germination of a spermatium is shown in Figure 4, B. Here is seen a gametophytic hypha grown up between the guard cells, with a deeply stained strand constricted between the guard cells. It is questionable whether the attenuated tip between the guard cells is a continuation of the hypha below or represents a spermatium which has elongated and is fusing with the hyphal tip. A number of similar cases have been seen. Hanna (25) reports the formation of germ tubes by spermatia of *Puccinia graminis*. These in one instance attained a length of  $15\mu$ . His opinion would seem to be that the spermatia germinate, penetrate the host tissue, and form uninucleate hyphae which grow into the aecial primordia and there fuse with fertile cells, thus initiating the binucleate condition. He does not state whether he has seen any evidence of such a procedure other than the slight germination of spermatia. The writer has obtained no evidence which would suggest that such a method of fertilization occurs in the bean or cowpea rust. A slight capacity for germination or elongation might be very useful in penetrating epidermal cells and making contact with gametophytic hyphae beneath the epidermis.

Another possibility suggested by Allen (2) is the fusion of spermatia with paraphyses extending through the ostiole of the spermogonium. Apparently it did not occur to her that these paraphyses, or some of them, might represent trichogynes. Her idea is that a "pynciospore" nucleus may pass down the paraphysis and join a second nucleus at the base of the "pyncium" to form a binucleate hypha which grows into the aecium. As a second possibility it is suggested that two associated spermatia may enter the paraphysis and form a binucleate hypha. She finds no continuous binucleate hyphae running between the spermogonium and the aecium, but does find scattered binucleate cells, which is exactly what should be found if these hyphae were functioning trichogynes. (Fig. 6, G, I.) As a matter of fact erumpent hyphae of the type described above are most frequently found closely associated with the spermogonia, often emerging at points very close to the ostiole. There is no obvious reason why they should not find an exit through the same break in the epidermis formed by the open spermogonium. An attempt was made to find this taking place in the forms studied here, but without success. There is considerable variation in the form of paraphyses protruding from the open spermogonia. Some are variously ruptured and vary in their nuclear content. Spermatia of course are always closely associated with

them, and one might easily imagine that a fusion is taking place in particular cases. But while it is possible that copulation of spermatia with trichogynes may take place at this point very frequently, it can not be said with certainty that it does. Such a concealed method of entrance might account very well for the fact that evidence of copulatory organs has been reported for so few species of rust in addition to the two under present consideration. It is probable, however, that hyphae penetrating the epidermis will be found in other rust fungi upon a study of material at the proper stage. Furthermore, it is not at all impossible that copulation may take place entirely within the host tissue. Such a phenomenon has been reported for *Collema pulposum* (Bernh.) Ach. by Bachmann (3). This would be especially feasible where the mycelium from sori of opposite sex has intermingled. Fusions under these conditions would be almost impossible to identify.

Some authors have attached a great deal of importance to the sterile cells in the aecium. To Christman (10) they were merely buffer cells which made room for a rapid growth of the fertile cells after fertilization. Blackman (7, p. 353) has interpreted them as vestiges of trichogynes. He says, speaking of *Phragmidium violaceum* Wint.:

Its [the sterile cell's] position above the fertile cell would suggest that it formerly acted as a receptive cell pushing up between the epidermal cells as a trichogyne to which the sticky spermatia could be brought, for example, by insects. Some support is lent to such a view by the fact that occasionally cases are to be found in which the sterile cells do push up between the epidermal cells and swell out above, being merely covered by the cuticle. If development were pushed one stage further and the cuticle pierced, a very effective receptive organ would be the result. [See fig. 7, L.]

Effective receptive organs are present in the two forms of *Uromyces* studied here, and they often protrude through the epidermis immediately above the aecium. (Fig. 5, F.) But these erumpent hyphae do not correspond to the sterile cells, but are, sometimes in addition to sterile cells, attached to the same fertile cell. These fertile cells with their hyphal branch reaching up to the host epidermis would correspond to the carpogones in the algae. They lie at all angles in the unfertilized aecium, as shown in Figure 2, B. Those nearest the epidermis become functionless as development proceeds below. It appears in this material that the sterile cells are not necessarily sterile but are fertile cells which may possess branches of their own and function as egg cells. As many as three of these are found in a chain. (Fig. 3, G.) The terminal cells are usually vacuolate and in part compose the pseudoparenchyma. Whether more than one fertile cell in such a chain ever produces a chain of aeciospores is uncertain, but they all appear to be essentially of the same structure. Figure 3, E, shows a linkage between basal cells, both of which are maturing spores. This is exactly the appearance that *a* and *b* of Figure 3, G, would probably have after each had begun to produce a chain of spores. It seems reasonable that the binucleate intercalary cell, which is present at the base of the mature aeciospore, corresponds to the secondary branch or trichogyne present on the uninucleate fertile cell. It is often observed that these intercalary cells are not interposed directly between aeciospores but are frequently at a sharp angle. (Fig. 3, D.) This may of course be entirely due to the pressure of surrounding cells.

A great deal of work has been done on the morphology of the aecium (23), but very little has been said of the structure of cells in the primordium and at the base of the aecium. Mention has been made of a

tangled mass of mycelium, but no one has seriously attempted to untangle the threads and to show their relationship to the enlarged fertile cells. Although she makes little comment, Allen (2) illustrates a condition which is found to be general in the primordia of bean and cowpea rust fungi. She shows hyphae with certain internodes greatly enlarged and supposes that these enlarged cells become the fertile cells. Since she saw no case of nuclei migrating into the fertile cells, she draws no conclusions as to the origin of the binucleate condition. Her drawings show 2-legged cells in the fertilized aecium, yet the fact that she saw no case of cell fusion would indicate that they are not fusion cells.

No observer previous to Allen has more than suggested the peculiar structure and arrangement of hyphae in the primordium. But in regard to the mature or fertilized aecium, they have been more exacting; although here, too, most investigators have failed to show the exact relationship between the basal cells and the hyphae underlying the hymenium. Figure 7, A, C, redrawn from Kursanov (29), and Figure 3, A, B, show essentially how the cells (a) of Figure 2, F and G, would appear after fertilization. It is obvious that the 2-legged basal cells shown in Figure 1, C, are of a similar origin—that they are not fusion cells but are a development from 2-legged cells present before fertilization.

In 1896, Richards (35) described what he termed "fertile hyphae" and "central bodies" found in the aecia of several rusts. (Fig. 10, A, B, C, redrawn from Richards.) No one since has been able to confirm his observations, although several have reported the presence of multinucleate cells and multinucleate hyphae in the aecia of various rust forms. Explanations which are reasonable enough have been offered for multinucleate structures. For example, it has been suggested that they represent the result of nuclear division having proceeded more rapidly than cell division. Figure 11 shows what Richards would probably have interpreted as "central bodies" and "fertile hyphae." Reference to his illustrations will show the similarity. This figure depicts an aecium with two centers or axes of development. In the section the two regions are distinguished from the rest of the aecium by a different intensity of stain and by their remarkable nuclear content. The cells at the upper end of one body show the beginning of a considerable proliferation, while in the other the walls separating it from adjoining cells have dissolved at two points. Walls between surrounding cells appear also to have broken down in some cases. Such a condition is not infrequent in the two forms studied, but is seldom as pronounced as in the case here represented. It probably occurs where conditions are unfavorable for fertilization. Stahl (37), Baur (6), and Darbishire (16) report that carpogones of *Collema* may disintegrate or return to a vegetative condition where spermatia are not found associated with the trichogynes. Bachmann (4) accounts for the absence of such disintegration in her form of *Collema pulposum* on the supposition that greater efficiency of the sexual mechanism results in every carpogone being fertilized. Colley (12) emphasizes the constant and normal occurrence of multinucleate cells at the base of the aecium in *Cronartium ribicola* Fischer. He suggests that in rusts of this type the aeciospore chains may arise more often from these placentalike cells than from basal cells resulting from a fusion of only two fertile cells. He con-

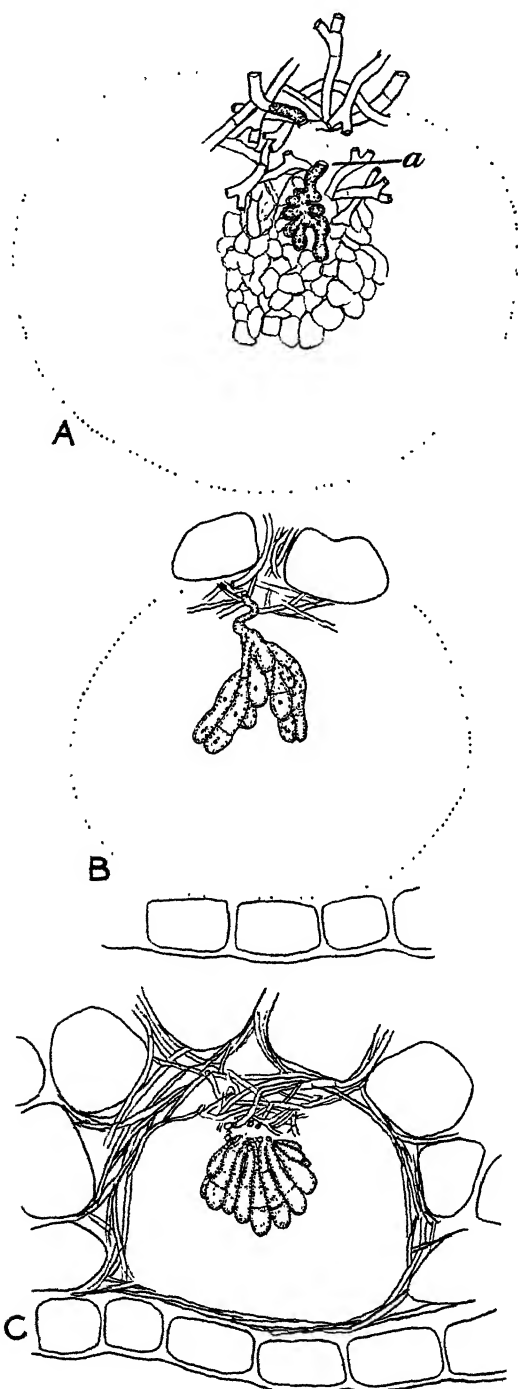


FIGURE 10.—Fertile hyphae and central bodies.  $\times 320$ . (After Richards, 35)  
For explanatory legend see opposite page

cludes that either the extra nuclei in these basal cells degenerate or such basal cells give rise to more than one chain of aeciospores. Basal cells often give rise by proliferation to several chains of binucleate aeciospores, but the extra nuclei in the basal cell do not represent an overdevelopment of a sexual process; they probably represent vegetative fusions and accompanying nuclear migrations in addition to the true fertilization through the trichogyne or trichogenous hypha. The resulting three or more nuclei may divide simultaneously, or two of them may divide while others appear inactive.

Figure 10, A, B, C, redrawn from Richards, and Figure 11, from material of the bean rust, may be interpreted as showing fertile cells which are in process of development while adjacent cells have failed to secure fertilization. The fertilized cells are developing at the expense of the others. The dissolved walls represent nutritive fusions, and the indefinite number of nuclei represent migrations which have in some cases accompanied cell fusion. The proliferated regions show the beginning of several chains of aeciospores which are supported by the increased food supply. It is easy to suppose that in particular cases only one egg cell in an aecium would become fertilized. In others, several would develop, while under very favorable conditions every egg cell would become fertilized, with the result that no proliferation would be seen and probably no cell fusion found.

There are numerous aberrancies among the Uredineae which may not seem to accord with the theory presented in this paper. As a matter of fact the facility with which many of these hitherto meaningless phenomena may be brought into accord by the present theory is most convincing evidence of its plausibility. Dodge has made a number of observations which are highly pertinent, and he has not entirely overlooked their possible significance. He observes the regular position of spermogonia of *Gallowaya pinicola* Arth. beneath stomata (20) and the more numerous development of stomata in the region of spermogonia of the orange rusts of *Rubus* (18). But most suggestive is his account of the formation of uninucleate aeciospores in the absence of spermogonia in one form of *Caeoma nitens* Burrill (19). There has been considerable difference of opinion as to the time relationship between the spermogonia and the aecia of some coniferous rusts. The observations of Weir and Hubert (38) on three species of *Cronartium* show conclusively the close association of the two spore forms and their probable functional relationship. In some other coniferous rusts it is well established that there is a 12-month interval between the appearance of spermogonia and the maturation of aecia (1). Such a delayed maturation following fertilization is not uncommon among fungi.

Some recent work on reproduction in the Ascomycetes (31) and in the lichens has brought into question the whole theory of fertilization by means of a trichogyne. If it should be proved that the so-called trichogyne in these forms does not function in reproduction, it is

#### EXPLANATORY LEGEND FOR FIGURE 10

[These illustrations have been redrawn and inverted]

A.—From an aecidium on *Ranunculus septentrionalis*. Fertile hypha (a), which has begun to form branches which will become aeciospore chains. Compare with Figure 11, d. B.—*Uromyces caladii*. Later appearance of proliferated basal cell. Compare with Figure 2, A. C.—From an aecidium on *Houstonia caerulea*. Proliferation here is from at least two centers

obvious that some of the arguments presented in this paper would lose much of their potency. Nevertheless, the writer has described what he believes to be the condition present in two forms of the Uredineae, which it is hoped to confirm and clarify by further research. If such should be the case, the presence of a sexual mechanism in the Uredineae similar to that reported for forms of Ascomycetes, lichens, and red algae should be accepted as additional confirmation of the trichogyne theory and an indication of the relationship among these four groups of organisms.

### CONCLUSION

The observations recorded here for two forms of *Uromyces* have shown that gametophytic hyphae grow up through the host epidermis and make contact with spermatia. The evidence is strong that spermatia pass into the ruptured hyphae. It has been shown that a

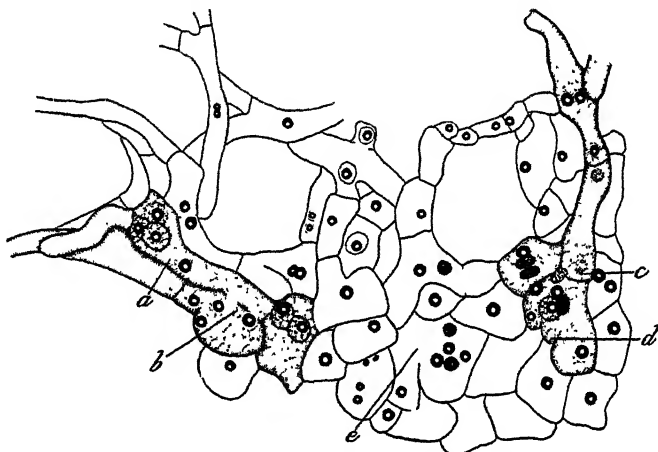


FIGURE 11.—Effect of delayed and incomplete fertilization in *Uromyces appendiculatus*. Section from aecial primordium showing fertilized cells at two centers (*a*, *c*), both representing a series of fertile cells. Vegetative fusions are shown at *b* and *e*, and an early stage in proliferation at *d*.  $\times 830$

receptive mechanism is present in the aecial primordium, and that nuclei migrate into the fertile cell through hyphae below and continuous with it. It is suggested that nutritive fusions may take place in a manner similar to those that occur on the carpogone of *Dudresnaya* (17, 34, 36). Fusion of adjacent cells in the Uredineae may therefore be compared to fusions with auxiliary cells in forms of red algae. The process of fertilization by fusion of equal gametes, as proposed by Christman (10, 11) and accepted by botanists generally, is brought into question, and the probabilities are that further study will prove the theory no longer tenable. It is obvious that details of the sexual mechanism and phenomena associated with the sexual process will vary with other forms, especially those rusts of the caeomoid type, and those forms, such as the Peridermiums, where the gametophyte is perennial in its host. It may well be that in some species parts of the sexual mechanism have degenerated and a substitute method of fertilization may have developed. In any case, doubtful features of the theory must be dealt with separately.

It is believed that the reasons which led Klebahn (28) to conclude that his stomatal hyphae were not functional have since been shown to be unobjectionable.

#### SUMMARY

A functional sexual mechanism similar to that found in the red algae is believed to be present in the gametophytic stage of the bean-rust organism, *Uromyces appendiculatus*, and the cowpea-rust organism *U. vignae*.

No evidence of the germination or fusion of spermatia has been obtained.

The 2-legged basal cells, similar to those called fusion cells by former observers, are present in the aecial primordium in the uninucleate condition previous to fertilization and are, therefore, not fusion cells.

This 2-legged structure is interpreted as being essentially an egg cell with a foot cell and a trichogyne.

The structures that are believed to function as trichogenous hyphae are much branched and highly septate organs having their terminus at the epidermis of the host leaf where they project through stomata or between epidermal cells and fuse with spermatia transferred by insects, by hand, or by other agencies.

Nuclei were found passing through the cross walls of these hyphae and migrating into the fertile cells in the aecium.

Fusions of parallel hyphae within the aecium do occur, but only under certain conditions, and are probably wholly vegetative or nutritive in function, and may precede, accompany, or follow the true fertilization.

Where all or most of the specially differentiated receptive cells in a single aecium become fertilized there is little or no budding or branching, but in case only one or a few egg cells are fertilized there may result an extensive proliferation which gives the appearance of central bodies and fertile hyphae.

Proliferation is made possible by nutritive fusions with unfertilized cells in the same aecium.

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# TWO WILD GRASSES AS HOSTS OF THE HESSIAN FLY, PHYTOPHAGA DESTRUCTOR<sup>1</sup>

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## INTRODUCTION

To cope with the Hessian fly most effectively it is essential to know all of its host plants. It is well known that this insect breeds to some extent in barley and rye, and the presence of what was apparently the true *Phytophaga destructor* Say in wild grasses has been reported occasionally. Webster<sup>2</sup> says:

Many years ago Lindemann, in Russia, found what he determined as the flaxseeds of the Hessian fly on timothy and *Agropyron repens*. Later Mr. Albert Koebele found flaxseeds closely resembling those of the Hessian fly on Elymus, Agrostis, Bromus, and Agropyron in California. Still later Mr. W. J. Phillips found similar flaxseeds in Agropyron about Richmond, Ind., May, 1908. During September of the same year Mr. E. O. G. Kelly found the Hessian fly breeding in abundance in *Agropyron smithii* in the vicinity of Wellington, Kans. \* \* \* On October 25 of the following year Mr. G. I. Reeves found many Hessian fly eggs and larvae in all stages of development up to the flaxseed on *Agropyron repens* and probably also on other varieties of this grass, growing up in the wheat fields in the vicinity of Vancouver, Wash., Mr. C. N. Ainslie found the flaxseed stage of the Hessian fly in *Agropyron tenerum* growing in alleys at Elk Point, S. Dak., June 23, 1913. There is therefore no doubt whatever but that the Hessian fly will breed in Agropyron and perhaps also, in some portions of the country, to a limited extent in Elymus, Agrostis, and Bromus.

Headlee and Parker<sup>3</sup> cite instances of the development of the Hessian fly in *Agropyron smithii*, *A. repens*, and *Elymus canadensis*. Hayhurst<sup>4</sup> found puparia resembling those of the Hessian fly in *A. repens* and reared adults from them. Progeny of these adults were bred from wheat and adults of both generations submitted to E. P. Felt, who stated that he saw no reason for not regarding all of them as the true Hessian fly. Osborn<sup>5</sup> discusses host plants rather extensively and is of the opinion that when forms resembling those of the Hessian fly are found in plants other than wheat their identity should be proven by biologic criteria. That is, in all of these reports there is an element of uncertainty as to whether the flies breeding in the grasses were the true Hessian fly. Osborn's opinion seems well taken. Biological observations are necessary to corroborate the taxonomic evidence that grass-infesting cecidomyiids are identical with *Phytophaga destructor*. If they could be reared from the grass, bred serially into wheat, back into the grass, and out again; or if *P. destructor* from wheat could be carried through a generation in the wild grass and a succeeding generation in wheat, proof of their identity would

<sup>1</sup> Received for publication Feb. 2, 1931; issued May, 1931.

<sup>2</sup> WEBSTER, F. M. THE HESSIAN FLY. U. S. Dept. Agr. Farmers' Bul. 640, 20 p. illus. 1915.

<sup>3</sup> HEADLEE, T. J., and PARKER, J. B. THE HESSIAN FLY. Kans. Agr. Expt. Sta. Bul. 188, p. 83-138, illus. 1913.

<sup>4</sup> HAYHURST, P. QUACK GRASS (AGROPYRON), A HOST OF THE HESSIAN FLY. Jour. Econ. Ent. 2 231-234, 1909.

<sup>5</sup> OSBORN, H. THE HESSIAN FLY IN THE UNITED STATES. U. S. Dept. Agr., Div. Ent. Bul. 16 (n. s.), 58 p., illus. 1898.

be complete. These processes have been completed experimentally in the greenhouse with *A. repens* and *E. canadensis*, and the object of this paper is to report the results.

### BREEDING EXPERIMENTS

In 1925 and 1926 W. B. Cartwright and the writer found larvae and puparia resembling those of the Hessian fly in *Agropyron repens* (L.) Beauv. and *Elymus canadensis* L.<sup>6</sup> near La Fayette, Ind. Attempts were made by the writer to rear succeeding generations in accordance with the procedure outlined above. Flies from these grasses were reared to the puparium stage on wheat, and flies from wheat were reared to the puparium stage on the grass. These rearings were made in outdoor cages, and the entrance of parasites prevented completion of the work.

During 1927 and 1928 the grass-infesting forms practically disappeared from the vicinity of La Fayette. Occasionally a small number of larvae or puparia were found in the wild grasses. Very few adults emerged from such material, and since both sexes were never obtained at the same time it was impossible to do any breeding. It is significant to note that in this same locality there was a coincident comparative scarcity of the Hessian fly in wheat.

Early in 1927 the writer undertook to rear progeny of Hessian flies from wheat and then breed them into *Agropyron repens* and *Elymus canadensis* in the greenhouse.<sup>7</sup> All plants were grown in 4-inch flowerpots to facilitate handling. Adults of *Phytophaga destructor* were reared from puparia taken from volunteer wheat and confined on the grass plants by means of cylindrical cages made of 18-mesh wire screen. They oviposited readily, after which they were removed from the cages and preserved. The young larvae seemed to experience some difficulty in becoming established on the grass plants. Consequently there was a higher mortality of this stage on the grasses than is common on wheat under similar conditions. However, the grass plants soon took on an appearance similar to that characteristic of fly-infested wheat. In 35 days larvae had matured and formed their puparia. The puparia were removed from the plants and placed on moist sand in small tin emergence boxes. Fifteen days later second-generation adults began to emerge and were caged on young wheat plants. After oviposition they were removed from the cages and preserved. The larvae hatching from these eggs established themselves readily on the wheat plants, matured, and formed puparia in an average of 27 days. The puparia were removed from the plants as soon as fully formed and placed on moist sand in tin emergence boxes. In the wheat-*A. repens*-wheat series third-generation adults, reared on wheat, emerged in about 15 days, and in the wheat-*E. canadensis*-wheat series adults of this generation emerged in about 10 days, thus completing the succession of generations on wheat, grass, and wheat. All adults of the third generation were preserved.

The adults of each generation preserved during the course of the experiment and labeled as to generation and host were submitted to C. T. Greene, of the taxonomic unit, United States Bureau of Entomology, who determined them to be *Phytophaga destructor*.

<sup>6</sup> Grasses determined by L. B. Lockwood, of the botany department of Purdue University.

<sup>7</sup> Greenhouse space was available through the courtesy of the Department of Entomology of Purdue University.

In November, 1929, the writer secured from *Agropyron repens* near La Fayette, Ind., 18 puparia resembling those of *Phytophaga destructor*. A few days later a similar find was made in *Elymus canadensis*, and an intensive search yielded about 2 dozen of the puparia. These were placed in emergence boxes and another series of rearings was begun. The method employed was essentially the same as with preceding greenhouse rearings. The flies from the respective grasses were caged separately on young wheat plants and left there until they had oviposited and died. They were then preserved for determination. Larvae hatching from these eggs established themselves readily on the wheat plants and produced a typical infestation in them. Second-generation adults emerging from wheat were in turn caged on young grass plants of the respective varieties from which their grandparents were taken, where they deposited eggs readily. After they had died they were preserved for determination. Larvae hatching from these eggs seemed to experience some little difficulty in becoming established on the grass plants, as was true in the wheat-grass-wheat rearings. However, enough of them did become established to produce a considerable infestation in the grasses, and were reared to the adult stage, thus completing the succession of generations on grass, wheat, and grass. All adults of this generation were preserved for determination.

All specimens preserved during the course of these rearings, labeled as to generation and host, were submitted to C. T. Greene, of the taxonomic unit, United States Bureau of Entomology, who identified them as *Phytophaga destructor*.

During the course of the experiments the question arose as to whether adult females known to be the Hessian fly would oviposit on the wild grasses if young wheat plants were available at the same time. To clear up this point, five pots were seeded with both wheat and *Agropyron repens* and five with both wheat and *Elymus canadensis*. When the plants were all in the 2-leaf stage adult flies reared from wheat were caged on them for oviposition. The flies were placed in the cages about 9 a. m. and left there until the following morning. They were then removed and all eggs counted. The results of the egg count are shown in Tables 1 and 2.

TABLE 1.—Eggs of *Phytophaga destructor* deposited on wheat and *Agropyron repens* in the same cages

Cage No.	Number of plants exposed		Number of plants infested		Number of eggs deposited on—		Average number of eggs per plant on—	
	Wheat	<i>A. repens</i>	Wheat	<i>A. repens</i>	Wheat	<i>A. repens</i>	Wheat	<i>A. repens</i>
1.....	4	5	4	5	42	94	10.5	18.8
2.....	6	7	6	7	233	161	38.8	23.0
3.....	6	5	6	5	53	94	8.8	18.8
4.....	5	6	5	6	260	278	52.0	46.3
5.....	5	5	4	5	212	124	42.4	24.8
Total.....	26	28	25	28	800	751		
Average per plant.....					30.8	26.8		

TABLE 2.—Eggs of *Phytophaga destructor* deposited on wheat and *Elymus canadensis* in the same cages

Cage No.	Number of plants exposed		Number of plants infested		Number of eggs deposited on—		Average number of eggs per plant on—	
	Wheat	<i>E. canadensis</i>	Wheat	<i>E. canadensis</i>	Wheat	<i>E. canadensis</i>	Wheat	<i>E. canadensis</i>
1.....	5	5	5	5	91	19	18.2	3.8
2.....	5	5	5	5	84	54	16.8	10.8
3.....	5	5	5	5	125	52	25.0	10.4
4.....	7	7	7	7	67	82	9.5	11.7
5.....	5	5	5	5	106	23	21.2	4.6
Total.....	27	27	27	27	473	230	-----	-----
Average per plant.....	-----	-----	-----	-----	17.5	8.5	-----	-----

Even with wheat plants available within the same small cages, no pronounced tendency of the flies to oviposit on the wheat in preference to the grasses was evident. However, it is possible that no great importance should be attached to this observation inasmuch as all oviposition by the Hessian fly in cages takes place under unnatural conditions.

#### SUMMARY AND CONCLUSIONS

That the Hessian fly (*Phytophaga destructor* Say) can subsist on wild grasses in addition to the cultivated small grains has been alleged for many years. Specimens which have been identified as that species have from time to time been reared from various wild or cultivated grasses, but it had not hitherto been proven that the forms thus derived could be bred back into wheat and afterwards returned to the original host plant. The results of the experiments reported in this paper show that this did occur. The conclusions drawn from this work are as follows:

The true Hessian fly (*Phytophaga destructor*) is able to complete its development on *Agropyron repens* (L.) Beauv. and *Elymus canadensis* L., although not so readily as in wheat.

This insect does sometimes breed in these grasses in nature.

The fly may, under favorable conditions, be able to subsist on these grasses in the absence of wheat.

# NUTRIENTS USED FOR MAINTENANCE BY GROWING DAIRY CATTLE<sup>1</sup>

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## INTRODUCTION

In connection with experimental studies in which immature dairy cattle are used the need frequently arises for information regarding the maintenance requirements of such animals. A search of the literature reveals a paucity of information on this subject and indicates the desirability of further investigation. Many studies relating to the maintenance requirements of cattle have been conducted since Henneberg and Stohmann (10, *v. 1, p. 17-188*)<sup>2</sup> reported their work in 1858. Important contributions in this field have been made by Wolff (16), Kühn and his coworkers (14), and Armsby (1).

A critical examination of the literature indicates, however, that as yet no comprehensive study has been made in which immature cattle were used as experimental subjects. Almost invariably mature animals have been used and most of them have been of the beef type. As a result, only one of the modern feeding standards, that of Armsby, even attempts to set forth the maintenance requirements of young dairy cattle. This standard was derived by calculation from results secured from mature beef animals, and its application to growing dairy cattle appears questionable. It would be expected that beef animals, because of differences in temperament and conditions of flesh, would offer a different problem from that of animals of the more active dairy type.

The influence of temperament on nutrient requirement was demonstrated by Armsby and Fries (2) in trials in which determinations were made over a 3-year period of the maintenance cost of a purebred beef animal and a scrub steer. The latter carried more or less Jersey blood and was of a decidedly more nervous disposition than the other steer. The results showed that the purebred steer required 5.623 therms of net energy for daily maintenance per 1,000 pounds live weight as compared to 6.141 therms required by the scrub steer under similar conditions. It has also been found that fattened steers have a greater maintenance cost per unit of surface area than do unfattened steers (3), suggesting a possible difference in this respect between animals of the beef and the dairy types.

The Armsby standard for maintenance of immature dairy cattle is founded on the principle that the net energy requirements of animals vary with the surface area rather than with the weight. Assuming that the net energy requirement at 1,000 pounds live weight is 6.0 therms daily, the requirements at the lower weights are calculated according to the following formula,  $X = 6.0 \left( \frac{W}{1,000} \right)^{.75}$ , in which  $X$

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<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 600.



is the maintenance requirement to be determined at the desired weight  $W$ , and 6.0 is the number of therms of net energy required for maintenance at a weight of 1,000 pounds. While the principle involved in this method is subscribed to by such workers as Kellner (13), Armsby and Moulton (4), and others, some investigators equally well known believe that the fundamental metabolism in animals is in nowise proportional to the body surface but is more nearly determined by live weight.

The question as to the applicability of the Armsby standard to dairy heifers in a practical way has frequently arisen. It was first brought to the attention of the writers as a result of a trial in which a heifer made a gain of approximately a pound a day for six months while receiving a ration the net energy value of which, when calculated by the Armsby method, was approximately the amount set forth as the maintenance requirement in Armsby's tables.

Furthermore, in a recent preliminary study of this problem by the writers (6) it was shown that the net energy necessary to maintain uniform weight in dairy-bred calves and heifers in normal flesh is about 90 per cent of that set forth in Armsby's tables. The number of trials represented in this study was too small, however, to afford conclusive results, and for this reason much additional data have been accumulated and are presented here with a more complete study of the problem.

#### EXPERIMENTAL PROCEDURE

The live-weight method of determining the maintenance requirement was adopted for this study. The many shortcomings of this method are fully appreciated. It is realized that maintenance in its strictest sense can not be determined in this manner. True maintenance, in which there is a perfect balance of matter, can be found only by complete balance trials in which either a respiration chamber or a respiration calorimeter are included as a part of the equipment. These methods, however, are complicated, entail much tedious work, and are very expensive. Even when it is possible to employ these more exact methods the number of animals that can be used is necessarily limited. The live-weight method makes up in part for the inaccuracies by the larger number of animals that can be used.

The chief errors encountered when the live-weight method is employed result from the tendency of the animal body to change in composition without a corresponding change in weight. It is recognized that less food nutrients may be required to maintain a growing animal at constant weight than to maintain actual equilibrium in the animal. The impulse to grow results in some growth taking place when live weight is maintained at a constant figure. Presumably fat oxidized from the tissues may be replaced by water. It is probable that when the trial period is of short duration—40 to 50 days—this possible error will not be sufficient to alter seriously the results obtained. The difficulty of securing accurate live weights is also well known and is a factor to be considered. The live-weight method has, however, in the past been considered sufficiently accurate to give usable results with mature animals in trials extending over long periods of time.

The procedure followed consists in estimating on the basis of a preliminary period, usually a week or more in length, the feed intake

needed for maintenance and then continuing this ration without change throughout the entire experimental period. If the ration as estimated proves to be more liberal than is required for maintenance, a gain will presumably be made until equilibrium is established. If it is too small, the same adjustment will occur at a point somewhere below the original weight.

The present study includes data from 19 animals of various ages used in 31 experimental periods. These periods ranged in length from 15 to 45 days, the average length being 32 days. With immature animals longer periods are believed to be impractical. The animals ranged in weight from 87 to 861 pounds. All were grade or purebred Holsteins. In all cases where an animal was used more than once, a sufficient interval was allowed between the trials to make certain that the subject was in a normal physical condition before it was placed on experiment the second time.

### RATIONS

Good quality alfalfa hay and wheat straw served as the sole sources of roughage. The grain ration used consisted of a mixture of ground corn, 4 parts; linseed meal, 1 part; wheat bran, 1 part. Skim milk was fed to the animals at the lower weights.

All feeds used except the skim milk were analyzed by the Division of Agricultural Biochemistry, and from these analyses the nutrient values were calculated by applying the digestion coefficients as given by Henry and Morrison (12). No effort was made to control the protein content of the ration except to provide what was considered an adequate supply at all times.

The animals were kept in individual stalls bedded with shavings and allowed the freedom of a dry lot during a part of each day when the weather was not too severe. Water was offered once daily in the barn and was always available in the barn lot. Salt and bone meal were kept in each stall. The weight of each animal was taken each morning before feeding.

### RESULTS

Table 1 is a summary of all experimental data. It shows the animals used, their weights, the rations fed during each experimental period, the length of each trial, and the nutrient value of the ration. The nutrient value is presented both in terms of net energy and total digestible nutrients.

As will be observed, two separate net energy values are given for each ration. The first of these represents the net energy content of the ration calculated by the method used by Armsby. The second value differs from the first in that a revised net energy value is applied to the alfalfa hay. Similar revised values for the other feedstuffs in the ration were not available. The revised net energy value applied to the alfalfa hay is the one determined by Forbes and Kriss (9) as a result of applying new and improved methods of calculation to the data already collected by Armsby. In this connection it is necessary to point out that Forbes and his coworkers (7) in recent studies have found evidence which indicates that the energy value of a ration depends to a great extent on the plane of nutrition at which it is fed. They state that in so far as the revised net energy values are concerned they are based on both submaintenance and supermaintenance feed-

ing, and in the light of present findings as mixed values they approximate "more closely values for body increase than values for maintenance." To date, however, these workers have not presented any definite net energy values based on this conception for feedstuffs when used for maintenance. The revised value suggested for alfalfa hay is therefore presented here tentatively until such time as a more accurate value becomes available. As would be expected, the revised net energy value of the alfalfa hay differs only slightly from that determined by Armsby, being a little higher.

TABLE 1.—Summary of data regarding weights and rations of 19 dairy calves used in maintenance trials

Animal No.	Weight of animal at—		Length of trial	Rations fed daily						Total digestible nutrients fed daily	Net energy in ration per day by—	
	Start of period	End of period		Alfalfa hay		Wheat straw		Grain mixture			Skim milk	Armstrong method
				Weight	Lot No. <sup>d</sup>	Weight	Lot No. <sup>d</sup>	Weight	Lot No. <sup>d</sup>			
	Lbs.	Lbs.	Days	Lbs.		Lbs.		Lbs.		Lbs.	Therms	Therms
E-79.....	87	87	20							4.0	0.65	1.16
E-129.....	110	114	35	1.00	23					6.0	1.06	1.25
E-87.....	114	114	30	.50	6					4.0	.91	1.33
E-128.....	125	128	35	1.00	23					7.0	1.15	1.35
E-79.....	135	135	25	1.00	6	0.50	2			7.0	1.36	1.41
391.....	233	232	40	1.50	6	1.00	2	0.50	A	5.0	2.02	1.75
390.....	249	247	45	2.00	6	1.00	2	.50	A	5.0	2.30	1.95
391.....	275	275	35	3.00	6	1.00	2	.50	A	5.0	2.83	2.29
F-54.....	284	282	40	2.00	3	1.00	1	1.50	5	6.0	3.13	2.84
390.....	308	309	15	3.00	6	1.50	2	1.00	A	3.0	3.23	2.48
E-15.....	323	325	35	5.33	1	1.25	1	.73	1		3.91	2.55
E-14.....	353	354	35	6.70	1	.74	1	.75	1		4.50	3.01
E-17.....	359	360	30	7.11	1	.81	1	.66	b	1	4.67	2.95
E-14.....	374	373	40	6.24	2	2.08	1	.95	2		4.85	3.12
E-16.....	394	394	40	7.38	1	1.10	1	.40	1		4.68	2.95
E-12.....	399	399	30	6.55	2	1.47	1	1.08	2		4.91	3.28
E-15.....	413	413	45	5.55	2	2.47	1	.53	2		4.30	2.57
E-65.....	416	415	35	7.00	5	1.00	2	1.00	b	B	4.67	3.11
E-55.....	455	455	40	7.00	4	1.50	2	1.00	B		4.95	3.21
E-53.....	462	461	20	6.50	4	1.00	2	1.50	C		4.94	3.43
E-54.....	468	470	40	7.00	4	1.50	2	1.00	b	B	4.95	3.21
E-16.....	521	519	30	7.00	2	2.50	1	.50	2		5.06	3.05
E-65.....	536	537	25	7.00	6	2.50	2	.50	b	D	5.08	3.15
E-46.....	588	592	25	9.50	5	2.00	2	1.00	B		6.31	4.01
E-55.....	593	595	25	9.25	5	2.10	2	1.00	B		6.21	3.94
E-53.....	663	664	25	10.25	5	2.10	2	1.00	B		6.71	4.25
415.....	675	678	20	10.50	23			c	2.00	16	6.75	4.95
E-46.....	798	799	40	10.50	6	2.50	2	1.50	D		7.74	5.18
411.....	833	837	30	12.00	23			c	2.00	16	7.52	5.46
412.....	840	837	25	12.00	23			c	2.00	16	7.52	5.46
E-53.....	861	863	35	10.00	6	3.00	2	2.00	D		8.06	5.49

<sup>a</sup> Whole milk.

<sup>b</sup> Feed of different lot number fed during part of period.

<sup>c</sup> Oats.

<sup>d</sup> The term "lot" is used to designate a certain quantity of feed sampled for chemical analysis. The analyses are given in Table 3.

Figures 1 and 2 present the distributions, indicated by dots, of the net energy provided by the rations as determined according to each of the two methods mentioned. For the sake of comparison Armsby's standard is shown by the solid line in both figures. Similarly the distribution of the total digestible nutrient value of the rations fed during each of the experimental periods is shown in Figure 3. The solid line in this figure indicates the maintenance requirements in terms of total digestible nutrients calculated from Haecker's (11) figures for the maintenance of mature cows, namely, 7.925 pounds of

total digestible nutrients per 1,000 pounds live weight.<sup>3</sup> The amount required at the various weights was determined according to the

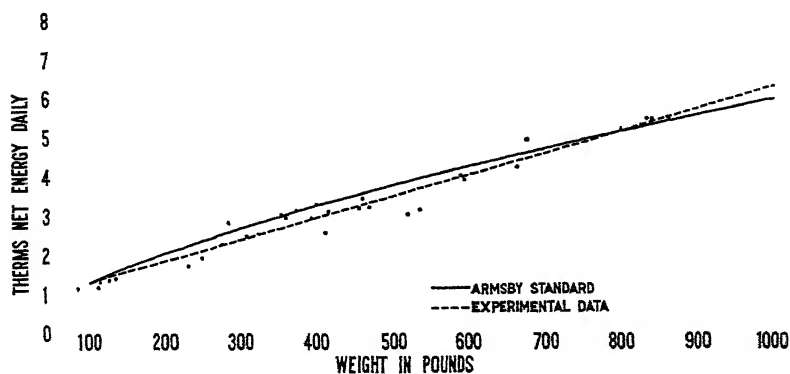


FIGURE 1.—Net energy value of experimental maintenance rations calculated by Armsby's method, compared with the Armsby standard for maintenance

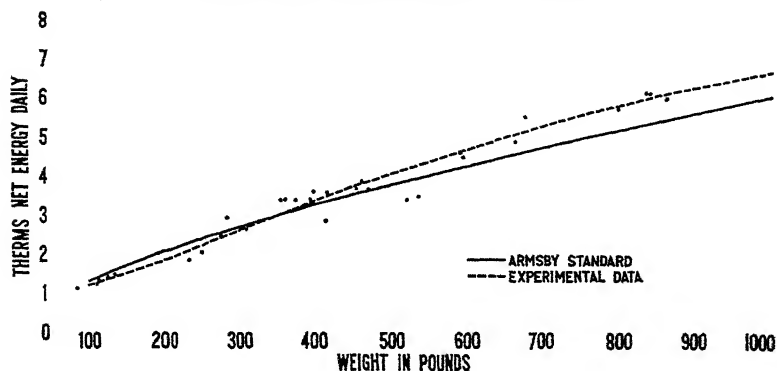


FIGURE 2.—Net energy value of experimental maintenance rations when revised value for alfalfa hay is used, compared with Armsby standard for maintenance

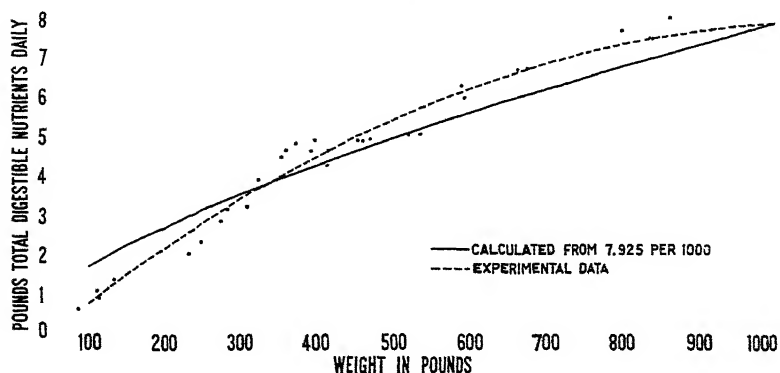


FIGURE 3.—Total digestible nutrients in experimental maintenance rations compared with requirements as calculated from Haecker's figure of 7.925 pounds at 1,000 pounds live weight

formula  $X = 7.925 \left( \frac{W}{1000} \right)^{2/3}$ , in which  $X$  is the maintenance requirement to be determined at the desired weight  $W$ , and 7.925 is the num-

<sup>3</sup> Morrison in his feeding standard for cows in milk makes use of this figure by Haecker.

ber of pounds of total digestible nutrients required for the maintenance of a cow at a weight of 1,000 pounds live weight. The values indicated by the dotted lines representing the distributions in each figure were determined largely by application of the method of least squares to the data.

TABLE 2.—Maintenance requirement of dairy calves in terms of therms net energy, and of total digestible nutrients, calculated from the experimental data, as compared with present maintenance standards

Weight (pounds)	Net energy required on basis of—			Total digestible nutrients required on basis of—	
	Armsby standard	Experimental data		7 925 pounds per 1,000 pounds live weight <sup>a</sup>	Experimental data
		Calculated by Armsby's method	Calculated by revised method		
	<i>Therms</i>	<i>Therms</i>	<i>Therms</i>	<i>Pounds</i>	<i>Pounds</i>
100.....	1.30	1.32	1.20	1.71	0.78
150.....	1.70	1.59	1.51	2.24	1.50
200.....	2.05	1.86	1.85	2.71	2.17
250.....	2.38	2.13	2.21	3.15	2.81
300.....	2.69	2.40	2.61	3.55	3.41
350.....	2.98	2.68	2.99	3.94	3.98
400.....	3.26	2.95	3.35	4.30	4.50
450.....	3.52	3.22	3.71	4.65	5.00
500.....	3.78	3.50	4.05	4.99	5.45
550.....	4.03	3.78	4.37	5.32	5.87
600.....	4.27	4.05	4.68	5.64	6.24
650.....	4.50	4.33	4.98	5.95	6.59
700.....	4.73	4.61	5.26	6.25	6.89
750.....	4.95	4.89	5.53	6.54	7.16
800.....	5.17	5.17	5.79	6.83	7.39
850.....	5.38	5.46	6.03	7.11	7.59
900.....	5.59	5.74	6.25	7.39	7.74
950.....	5.80	6.03	6.47	7.66	7.87
1,000.....	6.00	6.31	6.67	7.93	7.95

<sup>a</sup> This column is calculated by assuming the maintenance requirement to vary in proportion to the two-thirds power of the live weight. Figures varying directly with the live weight may be readily derived.

TABLE 3.—Chemical analysis of feedstuffs used in maintenance trials  
[Percentage on air-dry basis]

Feedstuff	Lot No. <sup>a</sup>	Moisture	Crude fiber	Crude protein	Ether extract	Nitrogen-free extract	Ash
Alfalfa hay.....	1	5.52	29.21	16.79	2.83	38.12	7.52
	2	7.08	25.27	16.12	2.84	40.58	8.10
	3	9.63	34.71	13.78	2.30	33.87	5.70
	4	7.11	28.11	20.44	2.31	32.15	9.88
	5	9.30	29.75	16.80	1.68	33.68	8.80
	6	7.06	33.29	15.40	2.11	36.65	5.49
Wheat straw.....	23	9.38	35.03	17.43	3.14	29.12	5.90
	1	7.30	37.13	4.56	1.69	39.97	9.35
	2	6.34	42.02	4.91	1.56	40.39	4.78
Corn.....	1	12.75	2.03	9.91	4.41	69.61	1.29
	2	15.34	2.33	9.88	3.76	67.25	1.44
	3	12.47	2.16	10.66	4.71	68.54	1.45
	4	11.46	3.05	12.37	3.71	67.77	1.65
Wheat bran.....	5	10.50	2.00	10.10	5.00	70.90	1.50
	1	11.65	8.34	14.63	4.66	54.38	6.34
	2	11.71	8.96	13.86	4.86	55.10	5.51
Oats.....	16	11.27	9.48	12.50	4.20	59.23	3.32
Linseed meal.....	1	9.71	8.84	34.89	6.25	35.52	4.87
	2	10.08	8.49	36.12	3.09	37.21	5.01
Whole milk.....		87.20		3.50	3.70	4.90	.70
Skim milk.....		90.10		3.80	.20	5.20	.70
Grain mixture.....	1	9.61	4.65	16.59	4.47	60.47	4.21
	2	8.82	6.28	15.81	5.45	59.00	4.63
	5	10.11	5.18	12.41	5.51	63.79	3.00

<sup>a</sup> See footnote d, Table 1.

Table 2 expresses the maintenance requirements based on the experimental data in terms of both total digestible nutrients and terms of net energy for immature dairy cattle calculated to regular weight intervals of 50 pounds. Table 3 gives the chemical analysis of all feedstuffs used in the trials and Table 4 shows the manner in which the various grain mixtures were prepared.

TABLE 4.—*Manner in which the grain mixtures were prepared*

Item	Lot number <sup>a</sup> used in making mixture—			
	A	B	C	D
4 parts corn.....	4	2	5	3
1 part wheat bran.....	2	1	1	1
1 part linseed meal.....	2	1	1	2

<sup>a</sup> See footnote d, Table 1.

### DISCUSSION

Table 1 shows that there is considerable difference in some cases in the requirements for maintenance of animals of approximately equal weights. These variations are perhaps no greater than would be expected in an experiment such as this in which so many factors are involved, many of which are difficult or impossible to control. The effect of difference in condition of animal alone may account for most of the variations observed. The importance of this factor was demonstrated in a previous study by the writers (6), in which it was found that animals in high condition may have a maintenance requirement as much as 30 per cent above that of normal animals. It has also been shown by others (5, 15) that after an animal has been on a low level of nutrition for a considerable period the requirements for maintenance are considerably reduced. Although, as has been stated, an attempt was made to have all animals in the same condition of flesh at the time of trial, some differences in this respect probably occurred, for, as is well known, it is very difficult to determine condition accurately in living animals. Other important factors contributing toward the variations indicated are differences in the nutrient value of the feedstuffs not shown by the chemical analyses and differences due to the individuality of the animal.

The recent work of Forbes and Kriss (9) previously referred to indicated that the net energy values as expressed by Armsby were on the whole somewhat too low. This finding is confirmed by the comparison of net energy values shown in Table 1. Using the revised net energy value for the alfalfa hay in the ration, the writers found that the requirements for maintenance under the conditions of the experiment were somewhat above those obtained by the Armsby method, especially at the higher weights. New values for the grain and straw would have but slight effect on the requirements of the older animals, as alfalfa hay forms the major portion of their ration.

The greater net energy requirements shown at the higher weights are probably due in part to the greater amount of exercise permitted the older animals. The younger animals while allowed some exercise in the open lot were confined in their box stalls to a greater extent.

It was also noticed that the younger animals showed a greater tendency to lie down and conserve their energy than did the older ones during the period of the trial. On the whole, considering the differences in methods and the character of the animals used, the data here presented show a remarkably close agreement with those obtained by the Armsby standard.

As would be expected when the nutrient values of the experimental rations are expressed in terms of digestible nutrients, similar tendencies are indicated as when net energy is used as the measure. A consideration of Figures 1, 2, and 3 seems to suggest that when the experimental data are expressed in terms of net energy they show a closer agreement with the standards used for comparison than when they are expressed in terms of total digestible nutrients. This is especially apparent at the lower weights. It is possible that the extremely low total digestible nutrient requirement at the lower weights may be due in part at least to a supplementing value of the milk, which formed a large part of the ration at that time. This is a fact not indicated in digestion tables. Moreover, the fact that the younger animals were perhaps more nearly in a state of perfect maintenance during the trials than the older animals might result in a more complete utilization of the nutrients provided. This is suggested by the studies of Forbes, Fries, Braman, and Kriss (8) in which the energy metabolism of animals on different planes of nutrition showed that the average rates of utilization of the net energy of the ration for maintenance and body increase were as 1 for maintenance to 0.761 for body increase.

#### SUMMARY

Experiments were made in which the live-weight method of determining the maintenance requirement of immature dairy animals was used, and the results from a total of 31 trials are reported. The results indicate that when the revised net energy value of alfalfa hay was used the requirements for maintenance under the conditions of the experiment were slightly lower than the requirement of the Armsby standard at weights below 350 pounds and considerably above it at the higher weights. When, however, Armsby's earlier method of calculating the net energy value of feedstuffs was applied to all the feedstuffs in the rations provided the amount of net energy received throughout agreed very closely with the requirements of the Armsby standard. The agreement is less close when the nutrients required and the nutrients received are expressed in terms of total digestible nutrients. In this latter case the comparison is made with Haecker's estimated maintenance requirement of 7.925 pounds of total digestible nutrients per 1,000 pounds live weight.

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# NUTRIENT REQUIREMENTS FOR NORMAL GROWTH OF DAIRY CATTLE<sup>1</sup>

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## INTRODUCTION

The study here reported was undertaken for the purpose of obtaining more accurate information regarding the nutrient requirements of growing dairy cattle. This is a problem that has claimed the attention of several investigators, and considerable information relating to it has been accumulated, especially in recent years; yet the knowledge in this field is still far from complete. It is apparent that not only is such information needed in connection with scientific studies relating to dairy cattle, but it is also of economic importance to the practical dairyman.

## PRESENT FEEDING STANDARDS

Feeding standards intended to serve as guides in feeding dairy heifers have been presented by Kellner,<sup>2</sup> Wolff-Lehmann as cited by Henry and Morrison,<sup>3</sup> Armsby,<sup>4</sup> Morrison,<sup>5</sup> and others. A careful analysis of these standards, however, discloses the fact that many of the data from which they were derived are of limited or doubtful value as applied to growing dairy cattle.

Kellner's standard is based on data secured from a few beef steers on rations composed of a limited number of feedstuffs. On the basis of present knowledge it seems apparent that the limited nature of the ration may have been a factor of sufficient importance to affect the accuracy of the results. Further questions may arise because of the small number of animals used and the possibility that beef steers differ from dairy heifers in their growth requirements.

The Armsby standard is based on records secured from a number of sources. Examination reveals that it was calculated by him largely from data taken by other investigators. Using as a basis the net energy value of a unit of gain, he accepts Soxhlet's<sup>6</sup> figure of 1.17 therms as the energy content of 1 pound of increase in live weight of a calf 1 month old. A uniform rate of increase is then assumed up to a maximum of 3.0 therms per pound of gain at 18 months of age. The standard was derived by applying these values to the actual gains secured in cattle at the Missouri station, both beef and dairy types, and adding to it the maintenance requirement. Armsby,<sup>7</sup> in expressing his opinion of it, says "it can not be claimed that the  
\* \* \* computations are particularly satisfactory. The data

<sup>1</sup> Received for publication Oct. 3, 1930; issued May, 1931. Paper No. 968 of the Journal Series of the Minnesota Agricultural Experiment Station.

<sup>2</sup> KELLNER, O. *THE SCIENTIFIC FEEDING OF ANIMALS*. Authorized transl. by W. Goodwin. 404 p. New York. 1913.

<sup>3</sup> HENRY, W. A., and MORRISON, F. B. *FEEDS AND FEEDING, A HANDBOOK FOR THE STUDENT AND STOCKMAN*. Ed. 18, 770 p., illus. Madison, Wis. 1923.

<sup>4</sup> ARMSBY, H. P. *THE NUTRITION OF FARM ANIMALS*. 743 p., illus. New York. 1917.

<sup>5</sup> HENRY, W. A., and MORRISON, F. B. *Op. cit.*

<sup>6</sup> ARMSBY, H. P. *Op. cit.*

<sup>7</sup> ARMSBY, H. P. *Op. cit.*, p. 402.

are scanty, and the element of personal judgment enters, especially into estimates of the energy value of a unit of increase in live weight."

The Morrison standard<sup>8</sup> is a modification of the Wolff-Lehmann standard, which was prominent a score or more years ago. According to Henry and Morrison,<sup>9</sup> the recommendations in the Morrison standard are based upon data from many sources, including some from European and some from American experiment stations.

It is recognized that the accuracy of any feeding standard can be determined only through its practical application. When present standards have been tested in this manner at this and other stations their inadequacy to serve as guides for feeding growing dairy cattle has been amply demonstrated. Further questions as to their ac-

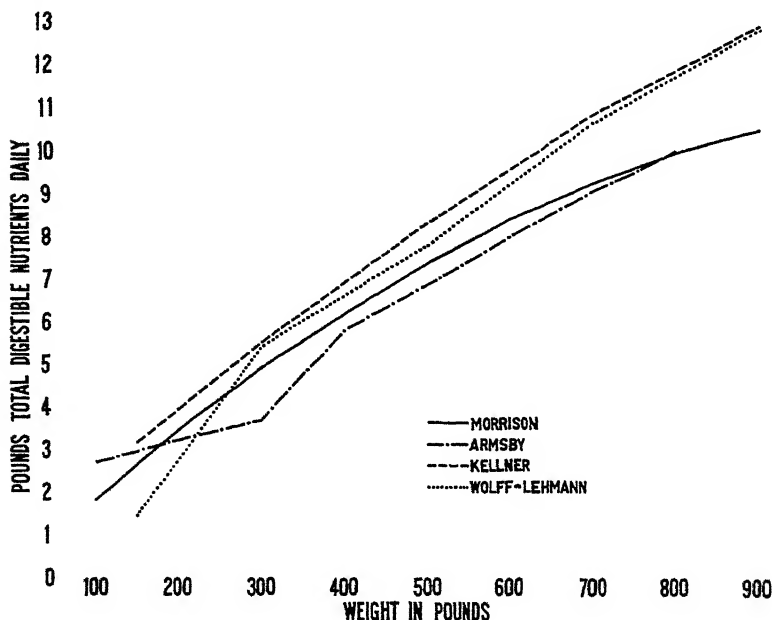


FIGURE 1.—Comparison of various feeding standards for growing dairy heifers; requirements expressed in terms of total digestible nutrients

curacy and practical applicability may arise from the general lack of agreement among them, which is apparent from a study of Table 1 and Figure 1. Since table and graph are self-explanatory a discussion of them will not be necessary. It should be mentioned, however, that the net energy requirements of Armsby and the starch values of Kellner have all been evaluated in terms of total digestible nutrients in order to make the comparison possible.

In the case of the Armsby standard, this was accomplished by selecting typical rations as used under practical conditions and making such small adjustments in the proportion of ingredients as found necessary to meet the requirements of the standard. This procedure was repeated for each 100 increment in weight from 100

<sup>8</sup> In Morrison's standard the recommendations regarding nutrients for an animal of a certain weight are given by two figures between which the requirement of the animal is expected to fall. In this paper the authors have used the average value of the range of these requirements for animals of different weights.

<sup>9</sup> HENRY, W. A., and MORRISON, F. B. Op. cit.

to 800 pounds. The value of these rations was then calculated in terms of total digestible nutrients by the use of the tables given by Henry and Morrison.

Kellner in his standard not only presents the requirements for growth in terms of starch equivalents but also in terms of digestible crude protein, fat, and nitrogen-free extract and crude fiber. The latter were combined by adding to the sum of the digestible crude protein, digestible nitrogen-free extract, and digestible crude fiber, the fat multiplied by 2.25 and expressing the result as "total digestible nutrients."

TABLE 1.—Requirements for growth of dairy heifers expressed in terms of total digestible nutrients as presented by various feeding standards

Weight	Total digestible nutrients required by the standard of—			
	Wolf-Lehmann	Kellner <sup>a</sup>	Armsby <sup>b</sup>	Morrison
Pounds	Pounds	Pounds	Pounds	Pounds
100			2.70	1.80
150	1.43	3.18	2.98	2.65
200	2.76		3.25	3.30
250	4.09		3.48	4.22
300	5.42	5.51	3.70	4.95
350	6.01		4.75	5.57
400	6.61		5.50	6.20
450	7.21		6.35	6.80
500	7.81	8.33	6.90	7.40
550	8.52		7.45	7.90
600	9.23		8.00	8.40
650	9.93		8.53	8.82
700	10.64	10.85	9.05	9.24
750	11.17		9.50	9.58
800	11.70		9.95	9.92
850	12.23			10.18
900	12.76	12.85		10.44

<sup>a</sup> KELLNER, O. *Op. cit.*, Table III, p. 393.

<sup>b</sup> Total digestible nutrient requirements at different weights determined by calculating typical rations according to Armsby net energy requirement and then evaluating these in terms of total digestible nutrients.

## PLAN OF THE EXPERIMENT

In determining the nutrient requirements for growth it is necessary to have a standard with which the growth of the animal may be compared, for obviously the level of feeding will be affected by the rate of growth. Such a standard has been presented by Eckles,<sup>10</sup> and in this study is used as the basis for comparison in all cases.

It is recognized that these normal-growth figures, especially for the Holstein breed, are somewhat low for conditions as maintained in many institution herds and by liberal feeders among purebred cattle breeders. On the other hand, Ragsdale and Regan<sup>11</sup> found that growing dairy animals on Missouri farms were smaller for their age than the figures given in the Eckles normal.

The experiment as conducted consisted of two parts. In the first, which was of a preliminary nature, the object was to determine whether or not the present feeding standards can be relied upon to serve as guides in feeding dairy heifers for normal growth. In this

<sup>10</sup> ECKLES, C. H. THE NORMAL GROWTH OF DAIRY CATTLE. Missouri Agr. Expt. Sta. Research Bul. 36, 23 p., illus. 1920.

<sup>11</sup> RAGSDALE, A. C., and REGAN, M. J. GROWTH AND DEVELOPMENT WITH SPECIAL REFERENCE TO DOMESTIC ANIMALS. XIV. (A) MEASUREMENTS OF GROWING HOLSTEIN AND JERSEY CATTLE ON MISSOURI FARMS. (B) PREDICTION CHARTS FOR THE GROWTH OF CATTLE. Missouri Agr. Expt. Sta. Research Bul. 142, 30 p., illus. 1930.

part of the investigation three groups of animals were used. The animals in these groups were provided with nutrients in amounts based on a definite percentage of their requirements according to the Morrison standard. Thus in Group 1 each animal received in its ration approximately 85 per cent of the required amount of nutrients, in Group 2 each received about 100 per cent, and in Group 3 each received approximately 115 per cent. The results were then measured in terms of growth as compared with the normal. Holsteins were used in all three groups, Jerseys only in the second. In the second and main part of the study the object was to determine the nutrients required to secure normal growth. This was accomplished by controlling the amount of nutrient received daily by each animal.

The experiment as conducted was extensive rather than intensive in nature. It was carried on over a period of almost 10 years and data were collected from nearly 50 animals. The animals used were purebreds or high-grade females of the Holstein and Jersey breeds. All animals were in normal health and vigor while on trial.

The ration fed throughout is believed to have been adequate in all essential respects. Young calves were fed whole milk until they were about 2 weeks old, the period of time varying with the vigor and physical condition of the animal. This was followed by skim-milk feeding until the calves were about 6 months old, when hay and grain became the sole source of nutrients. The standard grain mixture used throughout consisted of 4 parts corn meal, 1 part wheat bran, 1 part linseed meal (old process), all by weight. Good quality alfalfa hay was fed to all animals. Timothy hay and corn silage were used to supplement the legume hay in a few cases. The animals were never allowed to go on pasture.

#### CARE AND MANAGEMENT OF ANIMALS

The animals were kept in individual pens of a size that made it possible for them to be comfortable at all times. Shavings were used for bedding. Separate mangers were provided for the hay and grain and accurate records kept of the ration fed daily to each individual. Any feed refused was weighed and deducted from the amount fed during the period. Chemical analyses were made of all feeds and their nutrient value determined by using the digestion coefficients as given by Henry and Morrison.<sup>12</sup> Except during extremely cold or stormy weather the animals were allowed to exercise a few hours each day in the barn lot. Weights of all animals were secured every 10 days, and every 30 days weights were taken on three consecutive days, the average of the three being considered the weight for the period. Adjustments and changes in the ration fed were made whenever necessary, usually once every 30 days on the day following the 3-day weighing. As the protein requirement of the growing animal was not a part of this study, care was always taken to provide an adequate supply of it in the ration.

#### COMPILATION OF DATA

In making the compilations, the data from the preliminary portion of the study were divided into three classes. Class 1 included records from animals receiving 90 per cent or less of their nutrient require-

ment as prescribed by Morrison, class 2 included the records from animals receiving within 10 per cent; and class 3 those from animals receiving over 110 per cent of the amount of total digestible nutrients required. This plan was followed for each of the two breeds as far as they were represented in each class.

A uniform method of making the calculations was followed throughout. This consisted in calculating the amount of nutrients received by each animal as based on chemical analysis of the ration fed during each period. For animals up to approximately 6 months of age these periods were 10 days in length and each ended on the day the animals were weighed. Beyond this age, periods 30 days in length were used, each ending with the 3-day weigh period. The nutrient requirement during each of these periods, whether 10 days or 30 days in length, was based on the average weight of the animal during the period as determined by taking the average of the weight at the end of the period and that at the end of the previous period.

In all classes in both parts of the study a series of age intervals was established which ranged from 10 days for animals up to approximately 6 months of age to 30 days for the older animals. The average age, the average weight, and the average total digestible nutrients received daily were then used as representative of all the individuals included within each age interval of the series.

In the tables the data are arranged on the basis of regular weight intervals, showing the age of the animal and the total digestible nutrients received daily at the given weights. The total digestible nutrients received over maintenance per pound of gain in weight were derived by subtracting the maintenance requirement from the amount of nutrients received daily at a given weight and dividing the difference by the daily gain in weight. The maintenance requirement indicated for the various weights are those determined by the writers.<sup>13</sup>

## RESULTS

### CONTROLLED NUTRIENT INTAKE

The results from the trials in which the nutrient intake was controlled are presented in Tables 2-5 and in Figures 2-5. It will be observed that the amount of total digestible nutrients provided was approximately 15 per cent below, 100 per cent of, and 15 per cent above the Morrison standard for the animals in the three groups represented.

The most noteworthy fact concerning these data in view of the difference in the level of nutrition is the small variation in growth between the three groups during the first several months. This appears to indicate that within the limits of the experiment the rate of growth is not affected by the nutrient intake during this period. After this period the rate of growth of each group appears to be in proportion to the nutrient level. It is also apparent that the nutrient intake is sufficient for normal growth in each group up to the age of 6 to 8 months. Beyond this point there is a distinct tendency for the growth of both groups fed at the lower nutrient levels to drop below the normal for several months. The animals in the group on the lowest

<sup>13</sup> GULLICKSON, T. W., and ECKLES, C. H. NUTRIENTS USED FOR MAINTENANCE BY GROWING DAIRY CATTLE. Jour. Agr. Research 42: 593-601, illus. 1931.

level of nutrition showed this tendency most markedly, continuing below normal until more than 13 months old. It appears from these

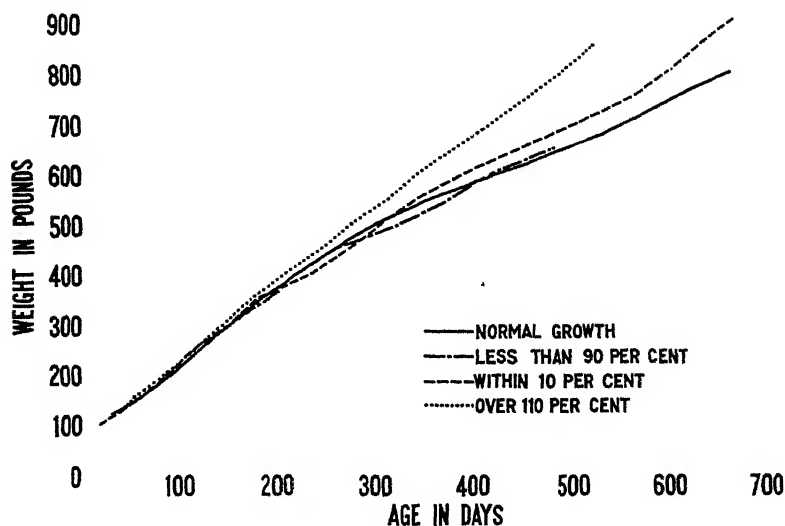


FIGURE 2.—Growth of Holstein heifers, on three planes of feeding, compared with the normal. The animals on the lowest plane of feeding received less than 90 per cent of their requirements as estimated by Morrison, those on the highest plane received 110 per cent or over, while the middle group received approximately 100 per cent.

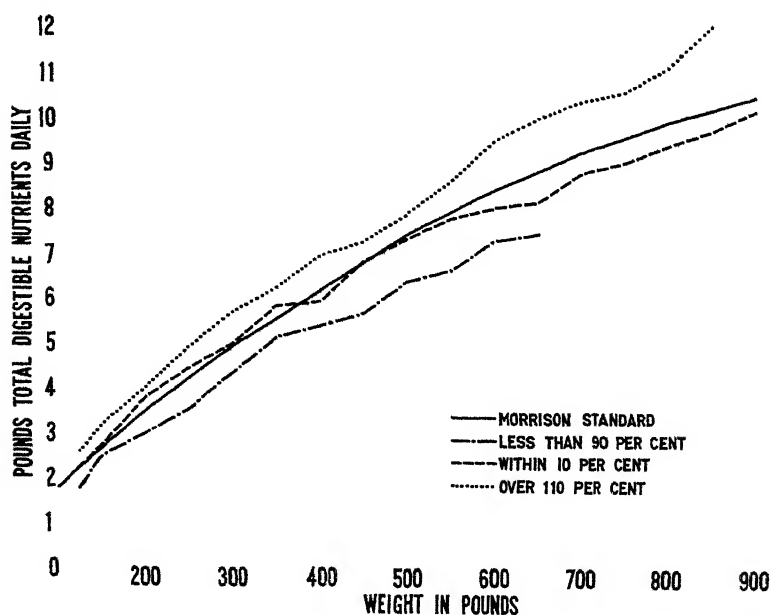


FIGURE 3.—Total digestible nutrients received by Holstein heifers on three planes of feeding. The Morrison standard is included for comparison. The animals on the lowest plane of feeding received less than 90 per cent of their requirements as estimated by Morrison, those on the highest plane received 110 per cent or over, while the middle group received approximately 100 per cent.

results that a nutrient level approximately that of the Morrison standard is adequate for normal growth in Holsteins up to about

6 months of age, after which it is slightly too low up to the twelfth month, and beyond this it is more than ample.

While the data for the Jerseys are limited to only one group, the results agree in all essential respects with those of the Holsteins. It

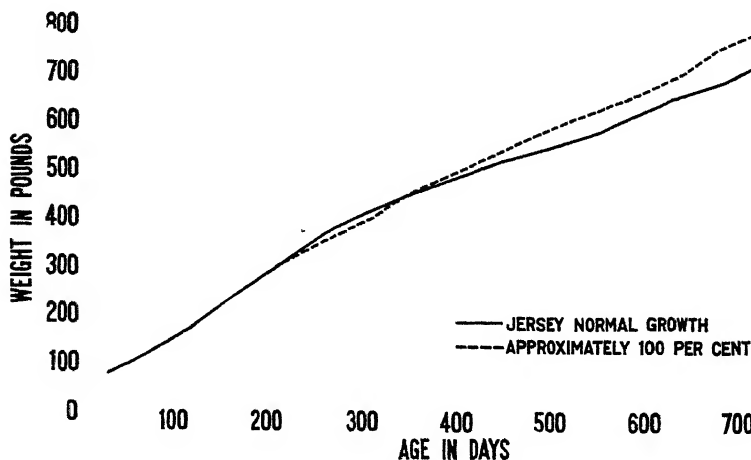


FIGURE 4.—Growth of Jersey heifers fed according to the Morrison standard compared with the normal

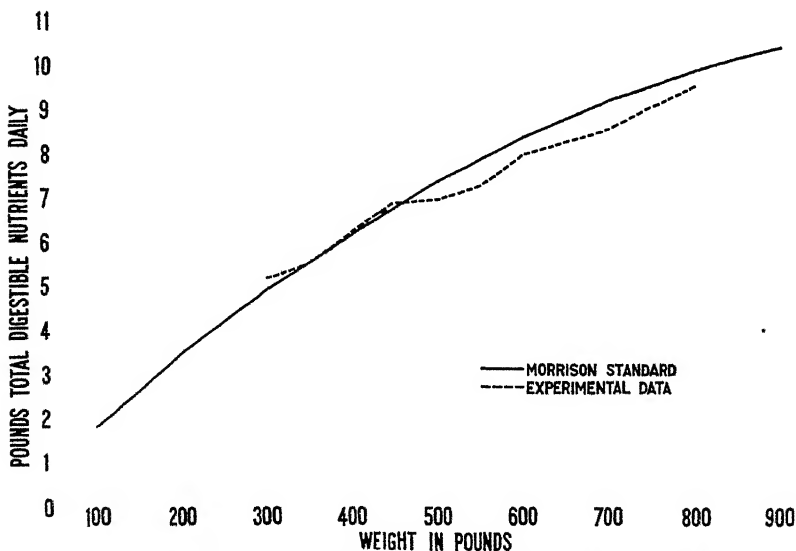


FIGURE 5.—Total digestible nutrients received by Jersey heifers fed approximately 100 per cent of their requirement according to Morrison. The Morrison standard is included for comparison

will be noticed that the nutrient level provided at ages above 1 year is actually nearer to the 90 per cent than to the 100 per cent level, indicating that the provisions of the standard are considerably too high during this period. This statement applies equally well to the Holsteins.



TABLE 2.—Weights, ages, nutrition planes, and maintenance requirements of Holstein heifers fed 90 per cent or less of their total digestible nutrient requirements as calculated from the Morrison standard

Weight	Age	Total digestible nutrients received per day	Plane of nutrition	Percentage of normal weight	Gain in weight per day	Total digestible nutrients required for maintenance	Total digestible nutrients over maintenance required per pound of gain in weight
Pounds	Days	Pounds	Per cent		Pounds	Pounds	Pounds
125	33	1.76	79.3	100.0		1.11	
150	54	2.45	92.5	100.0	1.19	1.50	0.80
200	89	3.00	85.7	100.5	1.43	2.17	.58
250	120	3.54	83.9	100.4	1.61	2.81	.45
300	149	4.36	88.1	100.0	1.72	3.41	.55
350	188	5.13	92.1	97.2	1.28	3.98	.90
400	216	5.41	87.3	101.0	1.79	4.50	.51
450	260	5.70	83.8	99.5	1.11	5.00	.61
500	324	6.39	86.4	95.6	.78	5.45	1.21
550	374	6.65	84.2	97.3	1.00	5.87	.78
600	418	7.29	86.8	100.8	1.14	6.24	.92
650	481	7.45	84.5	100.9	.79	6.59	1.09

TABLE 3.—Weights, ages, nutrition planes, and maintenance requirements of Holstein heifers that received approximately 100 per cent of their total digestible nutrient requirements as calculated from the Morrison standard

Weight	Age	Total digestible nutrients received per day	Plane of nutrition	Percentage of normal weight	Gain in weight per day	Total digestible nutrients required for maintenance	Total digestible nutrients over maintenance required per pound of gain in weight
Pounds	Days	Pounds	Per cent		Pounds	Pounds	Pounds
100	18.2	1.79	99.4	92.1	1.30	0.78	0.78
150	56.5	2.71	102.3	98.2	1.31	1.50	.92
200	85.5	3.80	108.6	103.4	1.72	2.17	.95
250	113.7	4.43	105.0	104.6	1.77	2.81	.92
300	149.2	5.02	101.4	100.0	1.41	3.41	1.14
350	176.8	5.84	104.8	101.7	1.81	3.98	1.03
400	233.5	5.95	96.0	95.9	.88	4.50	1.65
450	271.2	6.84	100.6	96.3	1.33	5.00	1.38
500	304.7	7.32	98.9	99.0	1.49	5.45	1.26
550	341.6	7.77	98.4	101.8	1.36	5.87	1.40
600	390.7	8.03	95.6	104.5	1.02	6.24	1.75
659	447.7	8.15	92.4	106.4	.88	6.59	1.77
700	507.2	8.78	95.0	106.3	.84	6.89	2.25
750	561.7	9.04	94.4	106.1	.92	7.16	2.01
800	596.7	9.40	94.8	107.8	1.43	7.39	1.11
850	625.4	9.72	95.5	110.5	1.74	7.50	1.22
900	664.9	10.16	97.3	112.4	1.27	7.74	1.91

TABLE 4.—*Weights, ages, nutrition planes, and maintenance requirements of Holstein heifers that were fed 110 per cent or over of their total digestible nutrient requirements as calculated from the Morrison standard*

Weight	Age	Total digestible nutrients received per day	Plane of nutrition	Percentage of normal weight	Gain in weight per day	Total digestible nutrients required for maintenance	Total digestible nutrients over maintenance required per pound of gain in weight
<i>Pounds</i>	<i>Days</i>	<i>Pounds</i>	<i>Per cent</i>		<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
125	38.5	2.58	116.2	95.4	2.07	1.14	0.80
150	50.6	3.16	119.2	102.7	1.51	1.50	1.23
200	83.8	4.03	115.1	104.5	1.66	2.17	1.26
250	114.0	4.90	116.1	104.5	1.68	2.81	1.38
300	143.8	5.72	115.6	103.0	1.89	3.41	1.20
350	170.3	6.25	112.2	105.0	1.38	3.98	1.78
400	206.5	6.96	112.3	104.2	1.36	4.50	1.66
450	243.3	7.26	106.8	104.8	1.59	5.00	1.50
500	274.7	7.83	105.2	106.0	1.33	5.45	2.04
550	312.3	8.59	108.7	107.3	1.61	5.87	2.02
600	348.4	9.49	113.0	110.8	1.40	6.24	2.42
650	379.1	9.94	113.2	114.4	1.27	6.59	2.71
700	418.6	10.33	111.8	117.6	1.42	6.89	2.40
750	453.8	10.57	110.3	121.7	1.44	7.16	2.58
800	488.6	11.10	111.9	123.5	1.60	7.39	2.79
850	519.8	12.05	118.4	127.1		7.59	

TABLE 5.—*Weights, ages, nutrition planes, and maintenance requirements of Jersey heifers that received approximately 100 per cent of their total digestible nutrient requirements as calculated from the Morrison standard*

Weight	Age	Total digestible nutrients received per day	Plane of nutrition	Percentage of normal weight	Gain in weight per day	Total digestible nutrients required for maintenance	Total digestible nutrients over maintenance required per pound of gain in weight
<i>Pound</i>	<i>Days</i>	<i>Pounds</i>	<i>Per cent</i>		<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
300	209	5.18	104.7	99.8	1.02	3.41	1.50
350	258	5.51	98.9	96.8	.96	3.98	1.83
400	310	6.26	101.0	96.3	1.25	4.50	1.52
450	350	6.90	101.5	100.4	.94	5.00	1.64
500	403	6.99	94.5	102.0	.80	5.45	1.61
550	459	7.30	92.4	105.0	.85	5.87	2.09
600	518	8.02	95.5	107.5	.75	6.24	1.99
650	585	8.08	91.6	106.6	.91	6.59	1.87
700	640	8.59	93.0	106.8	1.39	7.16	1.40
750	676	9.10	95.0	110.4	.81	7.39	2.69
800	738	9.57	96.5	109.8			

## CONTROLLED RATE OF GROWTH

The data regarding the animals of both breeds in which growth was controlled at the normal rate are presented in Tables 6 and 7 and Figures 6-9. The most striking feature definitely shown for both breeds is the tendency for the nutrient requirement to be above

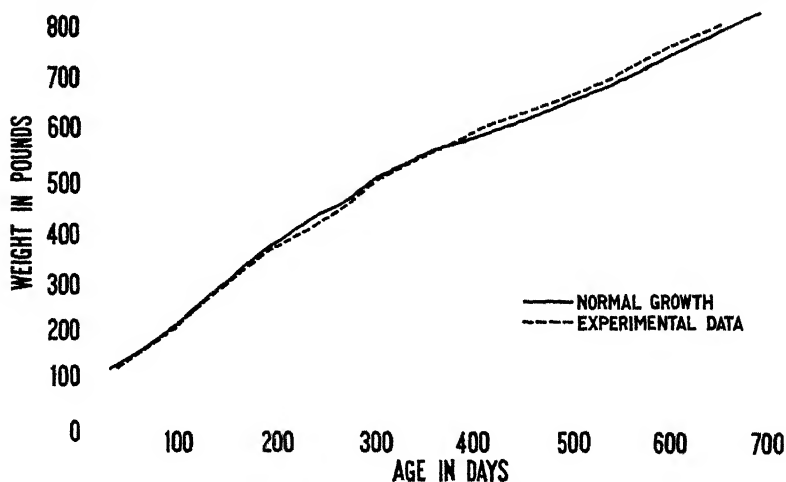


FIGURE 6.—Growth of Holstein heifers fed to grow at the normal rate. The normal growth curve is included for comparison

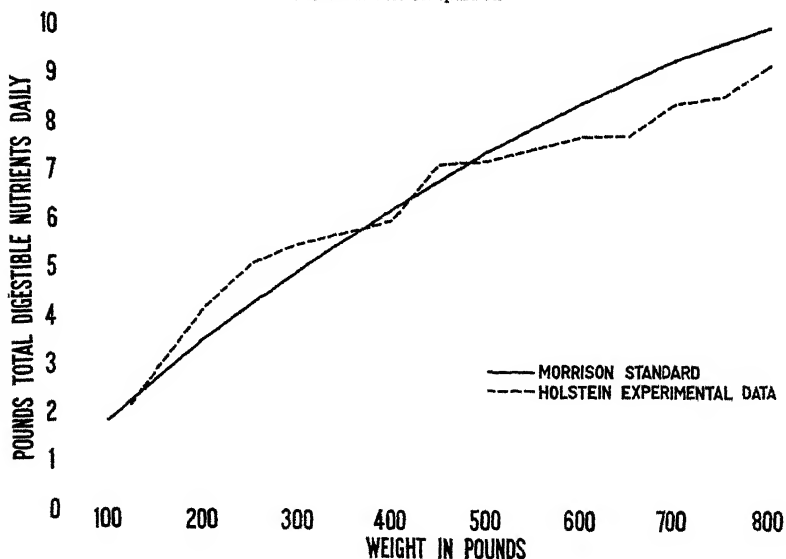


FIGURE 7.—Total digestible nutrients received by Holsteins fed to grow at the normal rate. Morrison standard is included for comparison

that of the Morrison standard for ages below 9 or 10 months. Beyond this point the provisions of the standard are more than ample for normal growth. These results tend further to corroborate what has already been suggested in connection with the preceding group, namely, that the provisions of the Morrison standard are somewhat

too meager for animals under 1 year old and excessive beyond this age.

The results show a tendency on the part of the Jerseys to have a somewhat higher nutrient requirement than the Holsteins at weights

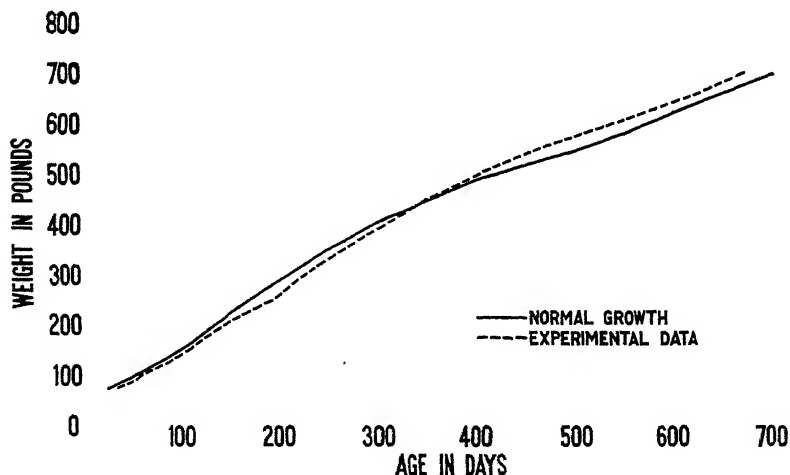


FIGURE 8.—Growth of Jersey heifers fed to grow at the normal rate. The normal growth curve is included for comparison

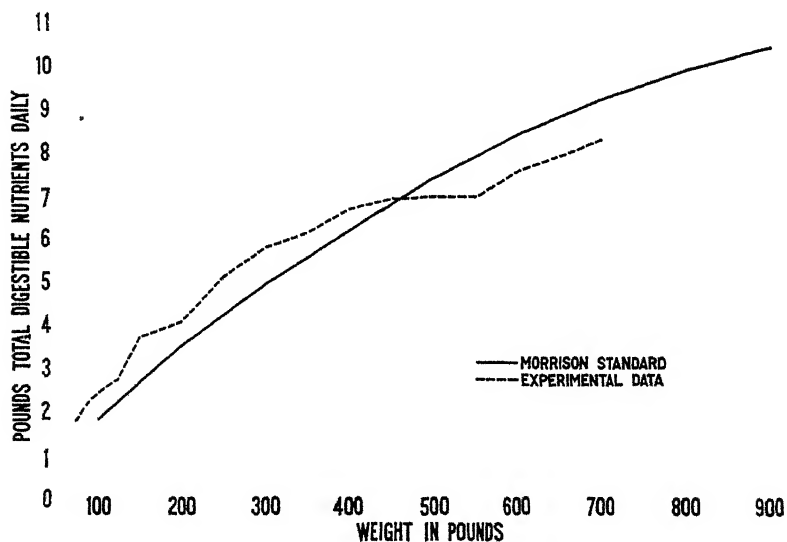


FIGURE 9.—Total digestible nutrients received by Jersey heifers fed to grow at the normal rate. The Morrison standard is included for comparison

up to about 200 pounds. Beyond this point, with few exceptions, the agreement is quite close between the two. This is clearly shown in Figure 10. It will be noted in Tables 6 and 7 that during this time the Jerseys were somewhat smaller with respect to the normal than the Holsteins, making heavier feeding possible as well as desirable.

Table 8 presents the estimated requirements for normal growth based upon the foregoing results from both Holsteins and Jerseys. Morrison's standard is included in the table for comparison. The same data are presented graphically in Figure 10.

#### NUTRIENT COST OF GAIN IN WEIGHT

It will be noted that the amount of total digestible nutrients required per pound of gain, beyond that needed for maintenance, is

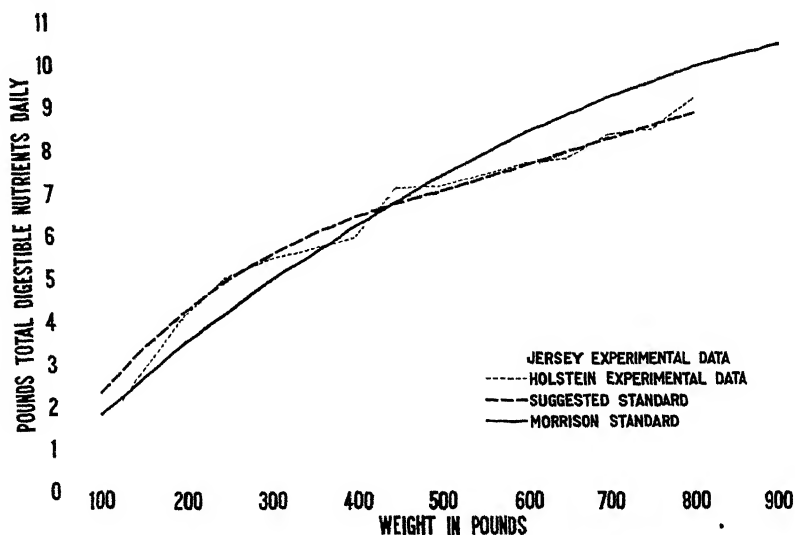


FIGURE 10.—Total digestible nutrients required for normal growth of heifers compared with the mean of the Morrison standard. The total digestible nutrients received by the Holstein and Jersey heifers for normal growth, upon which the suggested standard is based, are also indicated.

remarkably uniform for each group in both divisions of the study. There is, as would be expected, some indication, in several of the groups, of an increase in the amount of nutrients required with advance in age. On the other hand when comparisons are made between the various groups of the nutrient cost per pound of gain, considerable difference is observed. Considered in this way economy of gain appears to be correlated with the plane of nutrition provided.

TABLE 6.—*Weights, ages, nutrition planes, and total digestible nutrient requirements of Holstein heifers when their rate of growth was approximately 100 per cent of the normal throughout*

Weight	Age	Total digestible nutrients received per day	Plane of nutrition	Percentage of normal weight	Gain in weight per day	Total digestible nutrients required for maintenance	Total digestible nutrients over maintenance required per pound of gain in weight
<i>Pound</i>	<i>Days</i>	<i>Pounds</i>	<i>Per cent</i>		<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
125	38	2 15	96 8	95.7		1.14	
150	58	2.83	106 8	97.0	1 25	1.50	1.06
200	93	4 13	118.0	97.6	1 43	2.17	1.37
250	122	5.04	119.4	99.0	1.72	2.81	1.30
300	151	5.47	110.5	98.8	1 72	3.41	1.20
350	187	5 70	102.3	97 7	1 39	3.98	1.24
400	231	5.96	96.1	96.6	1.14	4.50	1.28
450	272	7.13	104.9	96.1	1 22	5.00	1.75
500	302	7.19	97.2	99.4	1.67	5 45	1.04
550	358	7.43	94.1	98 9	.89	5.87	1.75
600	408	7.70	91.7	102 2	1.00	6.24	1.46
650	479	7 71	87 4	101 2	.70	6.59	1.60
700	541	8.36	90.5	101.9	.81	6.89	1.81
750	588	8.48	88 5	102.2	1 06	7.16	1.25
800	650	9.18	92.5	101 4	.81	7.39	2.21

TABLE 7.—*Weights, ages, nutrition planes, and total digestible nutrient requirements of Jersey heifers when their rate of growth was approximately 100 per cent of the normal throughout*

Weight	Age	Total digestible nutrients received per day	Plane of nutrition	Percentage of normal weight	Gain in weight per day	Total digestible nutrients required for maintenance	Total digestible nutrients over maintenance required per pound of gain in weight
<i>Pound</i>	<i>Days</i>	<i>Pounds</i>	<i>Per cent</i>		<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
75	36	1.77	128 7	91.7			
90	53	2.24	137.4	91.7	0.88		
100	61	2.41	133.9	94.2	1.25	0.78	1.30
125	83	2.75	123.9	94.8	1.14	1.14	1.41
150	106	3.70	139.7	94.9	1 09	1.50	2.02
200	146	4.08	116.6	92.8	1.25	2.17	1.53
250	194	5.00	120.6	89.4	1.04	2.81	2.19
300	226	5.79	117.0	93.1	1.56	3 41	1.53
350	262	6 13	110.1	95 5	1.39	3.98	1.55
400	306	6.69	107.9	97.1	1.14	4.50	1.92
450	348	6.94	102.1	100.8	1.19	5 00	1.63
500	399	6.97	94.2	102.7	.98	5.45	1.55
550	461	6.97	88.2	104.8	.81	5.87	1.36
600	540	7.58	90.2	104.9	.63	6.24	2.13
650	611	7.92	89.8	103.0	.70	6.59	1.90
700	670	8.30	89.8	103.7	.85	6.89	1.66

TABLE 8.—*Total digestible nutrients found to be required for the normal growth of Jersey and Holstein dairy heifers as compared with the Morrison standard*

Weight	Total digestible nutrients received daily during experiments		Total digestible nutrients required daily by—	
	Jerseys	Holsteins	Experimental trial	Morrison standard <sup>a</sup>
Pounds	Pounds	Pounds	Pounds	Pounds
100	2.41	2.83	2.32	1.80
150	3.70	4.13	3.36	2.65
200	4.08	5.04	4.23	3.50
250	5.09	5.47	4.96	4.22
300	5.79	5.70	5.55	4.95
350	6.13	5.96	6.03	5.57
400	6.69	7.13	6.44	6.20
450	6.94	7.19	6.77	6.80
500	6.97	7.43	7.06	7.40
550	6.97	7.70	7.34	7.90
600	7.58	7.71	7.65	8.40
650	7.92	8.36	7.96	8.82
700	8.30	8.48	8.26	9.24
750	-----	8.18	8.56	9.58
800	-----	-----	8.85	9.92

<sup>a</sup> In the Morrison standard a range is given within which the requirement should fall. The figures used in this table are a mean of the two extremes as given by Morrison.

### SUMMARY

Comparisons are made between the Wolff-Lehmann, Kellner, Armsby, and Morrison feeding standards for growing dairy heifers. Experimental data are presented showing the nutrient requirements for normal growth of dairy heifers based on the normal-growth weights of Eckles, and also regarding the rate of growth of dairy heifers at different levels of the nutrient requirement as based on the Morrison standard.

The Morrison standard is found to be too low for normal growth of dairy heifers up to about 1 year of age and too high beyond that age.

The total digestible nutrient requirements found to be necessary for the normal growth of dairy heifers are shown.

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## SOME EFFECTS OF CHILLING TEMPERATURES ON SWEETPOTATOES<sup>1</sup>

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6. JULY 1931

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### INTRODUCTION

Sweetpotatoes (*Ipomoea batatas* (L.) Poir.) deteriorate rapidly after certain periods of exposure to temperatures ranging from  $-2^{\circ}$  C., the freezing point of the roots,<sup>2</sup> to about  $9^{\circ}$ . The obvious deterioration is due to fungi, but whether or not they are the primary cause was not known when the present investigation was undertaken (1920). There was a theory extant to the effect that sweetpotatoes were injured, independently of infection by microorganisms, when exposed to chilling<sup>3</sup> temperatures; but no data were available to support this theory.

A number of fungi, the more important being *Mucor racemosus* Fres., *Botrytis cinerea* Pers., *Alternaria* sp., and *Penicillium* sp., cause decay of sweetpotatoes<sup>4</sup> in from three to six weeks when stored at temperatures from  $0^{\circ}$  to  $8^{\circ}$  C. The temperature ranges in which these organisms have been shown<sup>4</sup> to cause decay are as follows: *Mucor racemosus*, from  $0.4^{\circ}$  to  $8^{\circ}$ ; *Botrytis cinerea*, from  $2.4^{\circ}$  to  $20.9^{\circ}$ , the highest temperature employed; *Alternaria* sp., from  $7.9^{\circ}$  to  $14.4^{\circ}$ ; and *Penicillium* sp., from  $4.4^{\circ}$  to  $12^{\circ}$ . These temperature relations were obtained from experiments in which the roots were inoculated with pure cultures of the organisms, in the case of *Mucor racemosus*, *Botrytis cinerea*, and *Penicillium* sp., by the "well" method;<sup>4</sup> and in the case of *Alternaria* sp., by inserting mycelium and spores into the root ends.

If sweetpotatoes are exposed to various temperatures without inoculation, the normal range for infection for all four organisms is about  $-1^{\circ}$  to  $9^{\circ}$  C. This normal temperature range for infection does not correspond with the temperature ranges for growth on culture media of any of the organisms. The temperature ranges for growth of *Penicillium* sp. and *Alternaria* sp. have not been studied, but both of these organisms grow readily at room temperatures ( $20^{\circ}$  to  $22^{\circ}$ ). The range of temperatures at which *Mucor racemosus* will grow on sweetpotato agar extends from approximately  $0^{\circ}$  to  $33^{\circ}$  (optimum about  $25^{\circ}$ ), and that at which *Botrytis cinerea* will grow on carrot agar is from about  $0^{\circ}$  to  $32^{\circ}$  (optimum about  $23^{\circ}$  to  $24^{\circ}$ ). There is also a

<sup>1</sup> Received for publication Nov. 1, 1930, issued May, 1931.

<sup>2</sup> WRIGHT, R. C., and TAYLOR, G. F. THE FREEZING TEMPERATURES OF SOME FRUITS, VEGETABLES, AND CUT FLOWERS. U. S. Dept. Agr. Bul. 1133, 8 p. 1923.

<sup>3</sup> As used in this paper, the term "chilling" applies to temperatures between the freezing point of sweetpotato roots (about  $-2^{\circ}$  C.) and  $9^{\circ}$ . These temperatures are usually regarded as unfavorable for the storage of sweetpotatoes.

<sup>4</sup> HARTER, L. L., WEIMER, J. L., and ADAMS, J. M. R. SWEETPOTATO STORAGE ROTS. Jour. Agr. Research 15: 337-368, illus. 1918.



discrepancy between the temperature ranges for growth and for infection of inoculated roots in the case of *Mucor racemosus*, *Penicillium* sp., and *Alternaria* sp. In the case of *Botrytis cinerea* no discrepancy is apparent. Although the range of temperature employed with this organism in the inoculation experiments did not extend above 20.9°, infection occurred at all the temperatures employed.

The foregoing data, particularly those relating to *Mucor racemosus*, indicate either that some change occurs in the roots at temperatures between the freezing point and 9° C. which makes them more susceptible to decay or that the pathogenes possess an ability to produce decay in sweetpotatoes at these temperatures that they do not have at higher temperatures. If this change in pathology as the temperature falls to 9° and below is due to the host, it may be regarded as the result either of injury or of a normal physiological process.

The purposes of the present investigation were (1) to determine whether it is possible, by modifying the humidity of the storage chamber at temperatures below 10° C., to prevent infection by the organisms listed above, and (2) to see if sweetpotatoes are injured at these temperatures.

#### MATERIAL AND EQUIPMENT

In the experiments conducted prior to the season of 1926-27 the Yellow Jersey variety of sweetpotatoes was used; subsequently, the Yellow Jersey Improved.<sup>5</sup> The sweetpotatoes were grown on the Arlington Experiment Farm, near Rosslyn, Va. They were cured and stored in a sweetpotato storage house constructed according to United States Department of Agriculture plans.<sup>6</sup> The curing temperature varied in different lots used, but the extreme variations were between 23° and 30° C. The subsequent storage temperature varied from 10° to 15°.

The roots were exposed to the chilling temperatures, some in cold-storage rooms 8 feet wide, 14 feet long, and 11.5 feet high, and others in galvanized-iron chambers 35 inches wide, 42 inches long, and 40 inches high, which occupied three of the cold-storage rooms. At the termination of the chilling period the roots, not examined for injury at the time, were stored in a sweetpotato storage house or in infection chambers described elsewhere.<sup>7</sup>

#### CONTROL OF TEMPERATURE AND HUMIDITY

The temperatures in all the chilling rooms, except those in which the chambers were installed, were maintained by means of brine refrigeration and controlled by hand. In addition to thermograph records, thermometer readings were taken twice a day for each room. The humidity of the two rooms in which the sweetpotatoes were chilled during the seasons of 1927-28 and 1928-29 was governed in one room by calcium chloride in evaporation pans and in the other by means of a commercial humidifier. The relative humidities of these two rooms were determined from psychrometer readings taken twice daily and from hygrothermograph records.

<sup>5</sup> LAURITZEN, J. I. A STRAIN OF YELLOW JERSEY SWEETPOTATO RESISTANT TO SURFACE ROT (FUSARIUM OXYSPORUM W. AND G.). Jour. Agr. Research 33: 1001-1004. 1926.

<sup>6</sup> THOMPSON, H. C. SWEET POTATO STORAGE. U. S. Dept. Agr. Farmers' Bul. 970, 27 p., illus. 1918.

<sup>7</sup> LAURITZEN, J. I., and HARTER, L. L. SPECIES OF RHIZOPUS RESPONSIBLE FOR THE DECAY OF SWEETPOTATOES IN THE STORAGE HOUSE AND AT DIFFERENT TEMPERATURES IN INFECTION CHAMBERS. Jour. Agr. Research 24: 441-456, illus. 1923.



Litho. A. Hoen & Co., Inc.

Sweetpotato roots exposed to a temperature of  $0.2^{\circ}\text{C}$ . for 45 days, showing brown areas  
(Painting by J. Marion Shull)



In the rooms where the galvanized-iron chambers were located the air was cooled by brine refrigeration and heated back to the desired temperatures by electric heating coils, and the temperature was controlled by mercury thermoregulators. The temperature of the chambers was the same as that of the room in which they were located. The humidity of the chambers was governed by means of calcium chloride and water in evaporation pans. Wet-bulb and dry-bulb readings were made through glass windows in the doors of the chambers from a psychrometer located just back of the windows. The necessary evaporation from the wet bulb was brought about by means of a fan blowing directly on the bulb.

#### EXPERIMENTAL DATA

The experimental work reported herein was carried on over a period of years extending from 1920 to 1929. The early work was limited because the desired conditions of humidity were not available. In the later experiments a variety of conditions of temperature and humidity became available and it was possible to isolate more definitely the effects of chilling temperatures.

A large number of trials made during the period extending from 1920 to 1922 and throughout the storage season showed that sweetpotatoes could be exposed to temperatures of 0°, 2°, and 4.5° C. for a period of 10 days and less without infection or apparent injury. Nor was infection or injury manifest after months of later storage in a sweetpotato house at temperatures ranging from 10° to 15°. In 1922 Gardner<sup>8</sup> reported experiments in which he exposed sweetpotatoes to temperatures as low as 0° for 12 hours without evident injury either immediately following chilling or after a period of storage at higher temperatures.

A large number of experiments conducted from 1922 to 1926 gave the same results consistently for the same temperatures and the same periods of exposure. Even when sweetpotatoes were stored at the freezing temperature of the roots (about -2° C.) long enough for some of them to freeze, the unfrozen roots, when subsequently stored for months at temperatures ranging from 10° to 15°, showed no apparent injurious effects.

Quantities of roots have been stored at temperatures ranging from 0° to 5° C. for 15 to 20 days in ordinary cold-storage rooms with very little infection or any evidence of injury that at the time could be dissociated from infection, either at the end of the chilling period or after a later period of storage at the usual storage temperatures. As the chilling period was extended beyond 20 days all the roots gradually became infected. It was not possible in the presence of such infection to determine whether or not there was injury. An effort was therefore made to see if it was possible to prevent infection by modifying the humidity of the storage chamber and thus permit of a study of possible injury.

During the storage season of 1926-27 the following nine combinations of temperatures and relative humidities were available: Relative humidities of 62, 80, and 96 per cent, each at temperatures of 9.5°<sup>9</sup> and 4.5° C., and relative humidities of 64, 79, and 95 per cent at 0.1°.

<sup>8</sup> GARDNER, W. A. SOME NOTES ON THE PHYSIOLOGY OF SWEETPOTATOES. Sweet Potato Bul. 2 (6): 7-10, 1922.

<sup>9</sup> The roots stored at 9.5° C. may be regarded as checks on the roots stored at the two lower temperatures. It would have been preferable to use a slightly higher temperature, because 9.5° is encroaching on the range of temperatures (below 10°) regarded as inimical to successful storage of sweetpotatoes.

Sixteen lots of 10 roots each of the Yellow Jersey Improved were stored in 4-quart till baskets at each of the above combinations of temperature and humidity. Two baskets were removed from each of the nine conditions at the end of 5, 10, 15, 20, 29, 40, 60, and 70 days. After the roots were examined for data on disease, one of the baskets from each condition was stored in a sweetpotato storage house (temperature 10° to 25° C.) for various periods. The uninfected roots from the other baskets were examined for internal injuries, and isolations were made from infected roots.

After various periods the stored half of the chilled roots were examined for the occurrence and amount of decay in the infected roots and for internal injury and percentage of germination in the uninfected roots.

#### INFECTION DATA

The data from the 1926-27 experiment regarding infection are recorded in Table 1. No infection by fungi that normally cause decay of sweetpotatoes at temperatures below 9° C. occurred at any of the relative humidities at 9.5°, during either the chilling periods or the subsequent storage periods. However, some infection with surface rot, caused by *Fusarium oxysporum* Schlecht. did occur. Infection by the fungi that normally cause decay at the chilling temperatures occurred at temperatures of 0.1° and 4.5° at all the humidities by the end of the chilling periods of 60 and 70 days, and at some of the humidities by the end of 20, 29, and 40 days. The full effects of the exposure to these temperatures for periods of 15 to 70 days were not manifest in some cases at the end of the chilling period, but became evident by the end of the subsequent storage period. It is certain that macroscopically invisible infections were incipient at the end of some of these chilling periods or that some infection occurred during the storage period, because not only was the percentage of infection larger in certain cases following the storage period, but it then became evident in some roots that had been subjected to the shorter chilling periods, thus having the effect of shortening the chilling period required for infection to occur.

No infection occurred after chilling periods of 5, 10, and 15 days at 0.1° and 4.5° C., and only 5 per cent at 4.5° after 20 days' chilling. No infection occurred after the storage periods subsequent to chilling in roots chilled for 5 and 10 days, and very little occurred in roots chilled for 15 and 20 days. Although there was no infection in 29 days of chilling at 4.5° with a relative humidity of 62 per cent, or in 29 or 40 days of chilling at 0.1° with relative humidities of 64 and 79 per cent, a rather high percentage of infection occurred in each case after a subsequent period of storage. After chilling for 60 and 70 days at all the humidities at temperatures of 0.1° and 4.5° all the roots were infected at the end of the storage periods. However, at the end of the chilling periods the percentages of infection were less in a number of these combinations of temperature and humidity than at the end of the storage periods.

TABLE 1.—*Influence of chilling temperatures and relative humidities on infection of sweetpotato roots by certain fungi*<sup>a</sup> (season of 1926-27)

Chilling temperature	Relative humidity	Total roots	Roots infected after indicated chilling period of days stated								
			5	10	15	20	29	40	60	70	
° C.	Per cent	Number	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
9.5	62	20	0	0	0	0	0	0	0	0	
9.5	80	20	0	0	0	0	0	0	0	0	
9.5	96	20	0	0	0	0	0	0	0	0	
4.5	62	20	0	0	0	0	0	25	80	60	
4.5	80	20	0	0	0	0	10	50	95	100	
4.5	96	20	0	0	0	5	30	90	100	100	
.1	64	20	0	0	0	0	0	0	40	95	
.1	79	20	0	0	0	0	0	0	30	75	
.1	95	20	0	0	0	0	5	75	100	100	
Roots infected after indicated chilling and storage <sup>b</sup> period of days stated											
			5 and 77	10 and 72	15 and 67	20 and 62	29 and 53	40 and 42	60 and 22	70 and 12	
			Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
9.5	62	10	0	0	0	0	0	0	0	0	0
9.5	80	10	0	0	0	0	0	0	0	0	0
9.5	96	10	0	0	0	0	0	0	0	0	0
4.5	62	10	0	0	10	0	40	50	100	100	100
4.5	80	10	0	0	0	0	80	70	100	100	100
4.5	96	10	0	0	0	0	90	100	100	100	100
.1	64	10	0	0	10	10	50	90	100	100	100
.1	79	10	0	0	0	0	50	70	100	100	100
.1	95	10	0	0	0	10	100	100	100	100	100

<sup>a</sup> Such as *Mucor racemosus*, *Botrytis cinerea*, *Alternaria* sp., and *Penicillium* sp.<sup>b</sup> After the various chilling periods the roots were stored for the periods indicated at a temperature of 10° to 25° C. and a relative humidity of 70 to 85 per cent. Second number indicates storage period

In roots chilled at a temperature of 4.5° C. the time required for the initial infection, as well as for infection of all roots, increased with the lowering of the humidity. In roots stored after they had become chilled at this temperature, the time required for infection to reach 100 per cent increased with the lowering of the humidity.

During the early stages of infection there was a direct correlation between the relative humidity and the percentage of infection in roots chilled, and in roots stored after they were chilled, at a temperature of 4.5° C., except when the roots were chilled for 15 days and stored for 67 days. In roots chilled at 4.5° but not stored there was an indirect correlation between the relative humidity and the period of time required to bring about infection. Such a correlation was not manifest in roots chilled at 4.5° and then stored.

In roots chilled at a temperature of 0.1° C., and in roots stored after chilling at this temperature, a relative humidity of 79 per cent was more effective in checking infection than one of 64 per cent.

The decay-producing organisms isolated from infected roots as they came from the chilling temperatures of 0.1° and 4.5° C. were *Mucor racemosus*, *Botrytis cinerea*, *Penicillium* sp., and *Alternaria* sp. Forms of *Fusarium*, in addition to the foregoing pathogens, were obtained from infected roots stored after they had been chilled at 0.1° and 4.5°. The invasion of *Fusarium* was apparently through the lesions produced by the chilling-temperature pathogens.

It was not possible by means of the relative humidities available to prevent infection by such organisms as *Mucor racemosus*, *Botrytis*

*cinerea*, *Penicillium* sp., and *Alternaria* sp. at temperatures of 0.1° and 4.5° C. However, it was possible to delay infection at these temperatures sufficiently to permit a study of the effects on the roots of longer exposures, aside from infection.

#### INTERNAL INJURY DATA

Throughout the entire period during which this work was conducted, 1920 to 1929, small percentages of the roots exposed longer than 20 days to temperatures of 0° to 5° C. have shown scattered areas of discolored tissue. These discolored areas, mostly brown (pl. 1) but sometimes tinged with black, were generally associated with the vascular tissue, but usually existed as local patches about the outer vascular tissue, rarely affecting it.

The results of the 1926-27 experiment are recorded in Table 2. Some symptoms of injury were found at all three temperatures, although only one root at 9.5° C. showed discoloration that may be attributed to the effect of this temperature, which is, of course, one-half degree lower than the minimum temperature (10°) recommended for the storage of sweetpotatoes.

TABLE 2.—Chilling temperatures in relation to internal injury of sweetpotato roots (season of 1926-27)<sup>a</sup>

Chilling period	Subsequent storage period	Roots chilled at—								
		9.5° C.			4.5° C.			0.1° C.		
		Total	Showing injury		Total	Showing injury		Total	Showing injury	
Days	Days	Number	Number	Per cent	Number	Number	Per cent	Number	Number	Per cent
5	0	30	0	0	30	0	0	30	0	0
10	0	30	0	0	30	0	0	30	0	0
15	0	30	0	0	30	0	0	30	0	0
20	0	30	0	0	30	0	0	30	0	0
29	0	29	0	0	29	2	6.9	26	1	3.8
40	0	30	0	0	23	2	8.7	14	0	0
60	0	29	0	0	3	3	100	14	0	0
70	0	30	0	0	6	1	16.7	3	1	33.3
5	77	30	0	0	30	0	0	30	0	0
10	72	30	0	0	30	0	0	30	1	3.3
15	67	30	0	0	29	0	0	29	1	3.4
20	62	30	0	0	30	0	0	28	2	7.1
29	53	29	1	3.4	9	0	0	10	4	40
40	42	13	0	0	8	1	12.5	4	2	50
60	22	8	0	0						
70	12	6	0	0						

<sup>a</sup> The data in this table were derived from the uninfected roots of the experiment from which the data in Table 1 were obtained.

<sup>b</sup> Thirty roots (10 roots from each of the 3 relative humidities) were used for each condition of time and chilling temperature, but in the final record only uninfected roots were counted in the internal injury data.

<sup>c</sup> At the end of the various chilling periods, the roots were stored for the periods indicated at a temperature varying from 10° to 25° C. and a relative humidity varying from 70 to 85 per cent.

The data obtained at temperatures of 0.1° and 4.5° C. are difficult to interpret because of the rare occurrence of the symptoms of injury in the form of discoloration and because of the erratic distribution of discoloration in relation to the treatment the roots received. Although there is a general tendency for the percentage of roots showing discoloration to increase with the increase in length of the chilling period, it is not marked, and there is one exception (compare the data obtained after 60 to 70 days of chilling at 4.5°). It is difficult to understand why the percentage of roots showing injury

should be larger in most instances at 4.5° than at 0.1° by the end of the chilling periods, when after a subsequent period of storage at 10° to 25° it was larger at 0.1° than at 4.5°. Since the total number of roots that showed symptoms of injury was small, it is possible that the distribution of the injury in the results obtained may have been influenced by the roots that were removed because of decay.

The shortest chilling exposure that resulted in symptoms of injury was 10 days at 0.1° C. This was in roots that were stored for 72 days after chilling. Otherwise signs of injury were not evident in roots chilled for only 10 days. Injury by chilling alone occurred after 29 days at both 0.1° and 4.5°.

The foregoing results were supplemented by two other experiments conducted on a limited scale during the storage seasons of 1927-28 and 1928-29. The results of these experiments are recorded in Table 3.

TABLE 3.—*Chilling temperatures in relation to internal injury of sweetpotato roots (seasons of 1927-28 and 1928-29)*

SEASON OF 1927-28					
Chilling temperature	Relative humidity	Chilling period	Subsequent storage period <sup>a</sup>	Total roots	Roots showing injury
° C.	Per cent	Days	Days	Number	Number
9.5	83	21	0	10	0
4.5	72	21	0	10	0
.7	64	21	0	10	0
.2	50	21	0	10	0
9.5	83	45	0	10	0
4.5	72	45	0	10	0
.7	64	45	0	22	0
.2	50	45	0	20	3
9.5	83	21	79	25	0
4.5	72	21	79	23	0
.7	64	21	79	25	0
.2	50	21	79	8	1
9.5	83	45	54	23	0
4.5	72	45	54	5	0
.7	64	45	54	10	0
.2	50	45	54	0	0

SEASON OF 1928-29					
Chilling temperature	Relative humidity	Chilling period	Subsequent storage period <sup>a</sup>	Total roots	Roots showing injury
° C.	Per cent	Days	Days	Number	Number
10	89	20	0	10	0
4.5	75	20	0	10	0
1.2	69	20	0	10	0
0	67	20	0	10	0
10	89	40	0	0	0
4.5	75	40	0	0	0
1.2	69	40	0	0	0
0	67	40	0	20	1
10	89	20	37	20	0
4.5	75	20	37	17	0
1.2	69	20	37	20	0
0	67	20	37	36	1
10	89	40	36	15	0
4.5	75	40	36	13	0
1.2	69	40	36	5	1
0	67	40	36	0	0

<sup>a</sup> The storage temperature and relative humidity were 21° C. and 90 per cent, respectively.

<sup>b</sup> The uninfected roots held at temperatures of 10°, 4.5°, and 1.2° C. for 40 days were not examined for injury until after 36 days of storage.

In the 1927-28 experiment, quantities of Yellow Jersey Improved roots were exposed for 21 days and 45 days to temperatures of 9.5°, 4.5°, 0.7°, and 0.2° C. and to relative humidities of 83, 72, 64, and 50



per cent, respectively. The subsequent storage temperature was 21° and the relative humidity 90 per cent. No injury was observed after 21 days of chilling. After 79 days of subsequent storage one root showed injury among those chilled for 21 days at 0.2°. Three roots showed injury after being chilled for 45 days at 0.2°. The roots chilled at 0.2° for 45 days were all decayed after 54 days of storage. No injury was evident in any of the roots chilled for 21 or 45 days at the other temperatures.

In the experiment conducted during 1928-29, roots of the Yellow Jersey Improved variety were chilled for 20 and 40 days at temperatures of 10°, 4.5°, 1.2°, and 0° C. and at relative humidities of 89, 75, 69, and 67 per cent, respectively. The subsequent storage temperature was 21° and the relative humidity 90 per cent. No injury was evident by the end of 20 days of chilling at any of the temperatures. After 37 days of subsequent storage, one root showed injury among those chilled for 20 days at 0°. One root was injured after 40 days of chilling at 0°. The roots chilled for 40 days at 0° and stored for 36 days were all decayed by the end of the storage period. One root showed injury among those chilled for 40 days at 1.2° and stored for 36 days. No injury was found at temperatures of 4.5° and 10° after any of the chilling or storage periods.

The association of a brown or sometimes slightly black discoloration with the exposure of sweetpotato roots to temperatures from 0° to 4.5° C. is not always constant, or consistent with the length of the exposure, but it is believed that it is to be regarded as a sign of injury due to chilling. (See pl. 1.)

#### GERMINATION DATA

In the germination tests the chilled roots were stored under conditions favorable for sprouting. In the 1926-27 experiment the roots were stored in a sweetpotato storage house during the latter part of the storage season (from April 6 to June 22, 1927), when the temperature ranged from 10° to 25° C. and the relative humidity from 70 to 85 per cent. That these conditions were favorable for sprouting is shown not only by the sprouting of the general stock, but by the sprouting of the roots stored after being exposed to 9.5° during the various periods of chilling. (Table 4.)

The number of roots sprouting at 9.5° C. varied from 90 to 100 per cent. The 90 per cent count was obtained after 70 days of exposure, but since there was no further relation of the percentage of germination to the length of the period of exposure it is believed that this slight drop in the percentage of germination in the longest period of exposure has no significance. There is a difference of only 3 per cent between the percentage of germination after 70 days and that after 15 days of exposure, one of the time intervals between these, however, yielding 100 per cent germination.

The percentage of germination varied from 93 to 100 in roots chilled at 4.5° C. for 5, 10, 15, 20, and 29 days, and dropped to 75 in roots chilled for 40 days. In this last case the percentage involved the absence of germination of only 2 roots out of 8, and it is not certain, therefore, that this drop has any significance.

In the roots chilled at 0.1° C., the number that germinated dropped from 90 per cent of those chilled for 15 days to 57 per cent of those chilled for 20 days, and to 0 per cent of those chilled for 29 and 40

days. These results seem to prove that roots exposed to a temperature of  $0.1^{\circ}$  for periods longer than 15 days are injured independently of infection, and to suggest a possible injury to roots exposed to  $4.5^{\circ}$  for 40 days.

TABLE 4.—*Influence of chilling temperatures on the germination of sweetpotato roots (season of 1926-27)* <sup>a</sup>

Chilling period	Subsequent storage period <sup>b</sup>	Roots chilled at—								
		9.5° C.			4.5° C.			0.1° C.		
		Total	Germinating		Total	Germinating		Total	Germinating	
Days	Days	Number	Number	Per cent	Number	Number	Per cent	Number	Number	Per cent
5	77	30	30	100	30	29	97	30	26	87
10	72	30	30	100	30	28	93	30	29	97
15	67	30	28	93	30	29	97	29	26	90
20	62	30	29	97	30	30	100	28	16	57
29	53	29	29	100	11	11	100	10	0	0
40	42	<sup>d</sup> 21	20	95	8	6	75	4	0	0
60	22	30	29	97						
70	12	20	18	90						

<sup>a</sup> The data in this table were derived from the uninfected roots remaining at the end of the experiment from which the chilling-period data in Table 1 were obtained.

<sup>b</sup> After the various chilling periods, the roots were stored for the periods indicated at a temperature of  $10^{\circ}$  to  $25^{\circ}$  C. and a relative humidity of 70 to 85 per cent.

<sup>c</sup> At temperatures of  $4.5^{\circ}$  and  $0.1^{\circ}$  C. each test was started with 30 roots (10 roots from each relative humidity), but in the final record only uninfected roots were counted in the germination data.

<sup>d</sup> Whenever the number of roots at  $9.5^{\circ}$  C. used in the counts was less than 30, the missing roots had been used previously for the purpose of making isolations from surface rot.

Germination data were obtained also in connection with the internal-injury data recorded in Table 3. These data are recorded in Table 5. After chilling, the roots in both experiments were stored at a temperature of  $21^{\circ}$  C. and a relative humidity of 90 per cent. The results in the main confirm those of the 1926-27 experiment. All the roots chilled at temperatures of  $9.5^{\circ}$  and  $10^{\circ}$  and those chilled at a temperature of  $4.5^{\circ}$  for 21 and 20 days in the 1927-28 and 1928-29 experiments, respectively, germinated. There was a drop from 100 per cent germination in roots chilled for 40 days at  $10^{\circ}$  (1928-29 experiment) to 77 per cent in those chilled at  $4.5^{\circ}$  for the same period. In the 1927-28 experiment, however, all the roots chilled at  $4.5^{\circ}$  for 45 days germinated. Thus, out of the three experiments (1926-27, 1927-28, and 1928-29), two (1926-27, Table 4, and 1928-29) showed a drop in the percentage of germination in roots chilled for 40 days at a temperature of  $4.5^{\circ}$ , while in the third (1927-28) there was no drop in germination in roots chilled for 45 days. In consequence of these inconclusive results, the question of injury in roots chilled at  $4.5^{\circ}$  remains uncertain.

The results given in Table 5 are consistent with those of the 1926-27 experiment (Table 4) in showing a drop in the percentage of germination below that obtained at  $9.5^{\circ}$  and  $10^{\circ}$  C. in roots chilled for 20 days and more at temperatures below  $4.5^{\circ}$ . The drop in the percentage germination in roots chilled for 20 days in the 1928-29 experiment was smaller than in those chilled for 21 days in the 1927-28 experiment. The difference in time of one day is not believed to be a factor. The decrease in the percentage of germination in the 1928-29 experiment would not in itself be convincing, but since it is consistent with all the other results, it is corroborative evidence of injury.

TABLE 5.—Influence of chilling temperatures on the germination of sweetpotato roots, seasons of 1927-28 and 1928-29

SEASON OF 1927-28						
Chilling temperature	Relative humidity	Chilling period	Subsequent storage period <sup>a</sup>	Total roots <sup>b</sup>	Roots germinating	
°C	Per cent	Days	Days	Number	Number	Per cent
9.5	83	21	79	25	25	100
4.5	72	21	79	23	23	100
.7	64	21	79	10	5	50
.2	50	21	79	8	0	0
9.5	83	45	54	23	23	100
4.5	72	45	54	5	5	100
.7	64	45	54	0	0	0
.2	50	45	54	0	0	0

SEASON OF 1928-29						
10	89	20	37	20	20	100
4.5	75	20	37	20	20	100
1.2	69	20	37	19	18	95
0	67	20	37	45	35	78
10	89	40	36	15	15	100
4.5	75	40	36	13	10	77
1.2	69	40	36	5	4	80
0	67	40	36	0	0	0

<sup>a</sup> The storage temperature and relative humidity were 21° C. and 90 per cent, respectively.

<sup>b</sup> The roots used comprise the uninfected roots remaining at the end of the experiments from which the data in Table 3 were derived.

### SUMMARY AND CONCLUSIONS

Sweetpotatoes of the Yellow Jersey Improved variety, when exposed for more than 20 days to temperatures ranging from 0° to 5° C. and relative humidities of 62 to 96 per cent, gradually became infected by such fungi as *Mucor racemosus*, *Botrytis cinerea*, *Penicillium* sp., and *Alternaria* sp. At temperatures from 0.1° to 4.5°, the percentage of infection was smaller (provided it had not reached 100 per cent) at the end of the chilling periods during which infection had occurred than after an additional period of storage at temperatures ranging from 10° to 25° C. Infection became evident during storage at a range of temperatures from 10° to 25° in certain cases where it was not evident at the end of the chilling periods.

In addition to the development of new lesions during the storage period, the old lesions became larger. These lesions apparently did not differ in appearance, aside from size, from those that developed during chilling. In addition to the above-mentioned fungi, forms of *Fusarium* were isolated from infected roots that had been stored at 10° to 25° C. after chilling at 0.1° to 4.5°. The invasion of *Fusarium* was apparently through the lesions caused by the other fungi.

During the chilling periods in which infection occurred, the percentage of infection in roots chilled at 4.5° C. was in general directly proportional to the relative humidity, as long as 100 per cent infection was not reached. At a temperature of 0.1° the percentage of infection was higher during its early stages at a relative humidity of 95 per cent than at the two lower humidities, both in roots only chilled and in roots stored for various periods after chilling. At a relative humidity of 79 per cent, however, there was less infection than at a relative humidity of 64 per cent.

The fact that sweetpotatoes are susceptible, at temperatures below 9° C., to infection which does not normally occur at temperatures above 9° may indicate a change in the physiology of the host or pathogenes, or it may mean that the host has suffered some injury. It seems probable that the change is in the host, since a simultaneous and parallel change in a number of pathogenes is hardly to be expected.

By lowering the relative humidity at temperatures of 0.1 to 4.5° C. from about 95 per cent to 80 and 62 per cent, it was possible to delay infection sufficiently to permit of an investigation designed to determine the presence and nature of any injury other than that caused by infection.

With the exception of one root which at 9.5° C. showed slight internal browning, suggestive of injury due to chilling, evidence of injury was not observed in roots held at temperatures ranging from 9.5° to 10°. Judging by internal discoloration, slight injury may occur in as little as 10 days' time in roots held at 0.1° and in 29 days in roots held at 4.5°. Only in the case of one root was injury observed in 10 days' time at 0.1°, after a subsequent storage period of 72 days. There is a tendency for the percentage of roots showing internal symptoms of chilling injury to increase with the length of the chilling period.

Evidence of injury was tested also by the ability of the chilled uninfected roots to germinate. In two experiments there was a drop in the percentage of germination in roots chilled for 40 days at 4.5° C., but in a third experiment no drop occurred after 45 days of chilling. Considering the small number of roots involved and the small drop in germination in the two experiments, together with the 100 per cent germination in the third, the evidence, while pointing to impairment of germination in roots chilled at 4.5°, remains, nevertheless, inconclusive.

In uninfected roots chilled at a temperature of 0.1° C. (1926-27 experiment) there was a decline in the percentage of germination, starting at the chilling period of 15 days, until there was no germination in roots chilled for 29 days. These results indicate that sweetpotato roots are injured when exposed to this temperature for 15 days or longer. The results of the germination experiments of 1927-28 and 1928-29 correspond closely with those of the 1926-27 experiment.

As a result of these findings, it would seem that the exposure of sweetpotatoes for periods less than 10 days to temperatures above the freezing point of the roots (-2° C.) and below 9.5° C. would not be injurious. A prolonged exposure, however, would subject the roots not only to the danger of infection by the various low-temperature fungi but also to chilling injury that may or may not be evident at the end of the chilling period. Such signs of injury may become evident after a period of storage even in a temperature favorable to sweetpotatoes.

Taking into consideration the danger of both chilling injury and infection, there is nothing to commend even the temporary exposure of sweetpotatoes to temperatures below 10° C.



# EFFECT OF ABNORMALLY LONG AND SHORT ALTERNATIONS OF LIGHT AND DARKNESS ON GROWTH AND DEVELOPMENT OF PLANTS<sup>1</sup>

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## INTRODUCTION

One of the striking facts developed in the study of plant response to relative length of day and night is that, so far as concerns initiation of flowering and fruiting, darkening the plant for a period in the middle of the day fails to produce the same effects as those resulting from excluding the early morning or late afternoon light. Early in the investigations of the present writers on this subject<sup>2</sup> it was shown that in such typical short-day plants as Biloxi soybeans (*Soja max* (L.) Piper) and *Aster linariifolius* L., darkening from 10 a. m. to 2 p. m. each day has but little effect in hastening flowering as compared with that produced by exposure to an unbroken short day. Further studies have shown that other short-day plants, as well as typical long-day plants, also show a curious indifference to darkening in the middle of the day with respect to flowering and fruiting, even when the amount of light thus excluded is vastly greater than that excluded with decisive effects in the early morning and late afternoon. These results indicate that, with a given total number of hours of daily illumination, two short periods do not produce the same result as a single unbroken period of illumination. Indeed, the effect of mid-day darkening is much the same as if the plants remained in the light for the whole day.

Another prominent feature of photoperiodic response in plants is that species and varieties differ widely in their sensitivity to change in length of day. Comparatively small changes in day length may exert marked formative action on some plants, while in other plants wide variation in the daily light period may produce only slight quantitative effects. The question naturally arises as to the character and extent of the effects that would be produced on the two classes of plants by alternations of light and darkness falling outside the range commonly experienced in nature. To obtain further information on this problem, with the results previously obtained by darkening soybeans and *Aster* in the middle of the day as a basis, the experiments discussed in this paper were undertaken. These experiments have been carried out on a number of plants of long-day, short-day, and indifferent types, with sunlight and with the tungsten-filament lamp as sources of illumination. A preliminary account of some of the earlier, less comprehensive tests appeared in 1927.<sup>3</sup>

<sup>1</sup> Received for publication Jan. 2, 1931; issued May, 1931.

<sup>2</sup> GARNER, W. W., and ALLARD, H. A. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. *Jour. Agr. Research* 18: 553-606, illus. 1920.

<sup>3</sup> GARNER, W. W., and ALLARD, H. A. EFFECT OF SHORT ALTERNATING PERIODS OF LIGHT AND DARKNESS ON PLANT GROWTH. *Science* (n. s.) 66: 40-42. 1927.

## METHODS OF PROCEDURE

Efforts were first directed toward amplifying the original results obtained with soybeans and Aster by testing other plants and by employing several modifications of the light treatment. It was desired to obtain as many data as possible with sunlight, bearing on the effects of alternations of light and darkness not commonly experienced by plants in nature, in order to furnish a check on the results obtained with artificial light. However, the opportunities for the use of sunlight in securing these alternations are rather limited because of the inevitable sequence of day and night in a cycle of 24 hours. In one series of tests the plants were subjected alternately to a 10-hour day and to the full day length of summer. For this and the two following series of tests with sunlight, the usual arrangement of light-proof houses and trucks on steel tracks for transferring the plants was employed. The midsummer day length at Washington, D. C., from sunrise to sunset, is slightly less than 15 hours.

In another series of tests conducted in midsummer the plants were darkened throughout the daylight period and exposed to full-day illumination on alternate days. In this way the alternations of light and darkness involved a cycle of 48 hours instead of the usual 24 hours. The plants were illuminated for about 15 hours in each 48-hour period, which would correspond to an average of  $7\frac{1}{2}$  hours of light daily. Along with these tests, another treatment involved daily darkening from 10 a. m. to 3 p. m. In this case the plants were illuminated for an average of about  $9\frac{1}{2}$  hours in each 24-hour period. In two instances the darkening was from 10 a. m. to 2 p. m. daily.

In the final series of tests with sunlight the plants were placed in the light-proof houses from 10 a. m. to noon and from 2 to 4 p. m. Thus they were exposed to three short periods of illumination each day, and the total daily illumination ranged from 11 hours to about 9 hours or slightly less, depending on the duration of the test.

To obtain systematic alternations of light and darkness of fixed duration it is necessary, of course, to employ artificial light. For the tests with artificial light a series of small light-proof compartments was used, each compartment being provided with a 1,000-watt Mazda lamp, with reflector, as a light source. The plants were protected from excess of radiant heat from the lamps by interposing a 2-inch screen of distilled water which was constantly recirculated from a tank by means of an electrically driven pump. A current of air was driven through the plant chambers at a uniform rate by means of a fan. The lights were turned on and off automatically at definite intervals by passing the electric current through a series of revolving drums having a segment cut away and fitted with copper brushes so as to provide for making and breaking the electric circuit. The time relationship between complete revolutions of the different drums was rigidly maintained by means of a series of gears, the whole being driven by a synchronous electric motor.

Except in the final tests, means were not at hand for maintaining constant temperature and humidity. Each series of tests, however, was essentially complete in itself, and thermographic records indicate that in each series the difference in the temperature of the different compartments was not at any time more than 1 or 2 degrees Fahrenheit. A fairly large number of experiments were carried out at

various temperatures and on various plant species. As an additional check, in some instances the alternations of light and darkness allotted to the different compartments were interchanged, in order to eliminate the effects of abnormal conditions that might exist in any compartment. In each series the mean temperatures were computed from the thermographic records. In the shorter alternations of light and darkness no changes in temperature due to the light coming on or going off could be detected. In the alternations of one hour or longer a rise of 1 or 2 degrees in temperature usually resulted when the lights came on. In the final tests a system of automatic control of temperature and humidity was in operation and, except on abnormally warm days, the temperature was held constant within  $\pm 2^{\circ}$  F., while the relative humidity was held constant within about  $\pm 3$  per cent.

By means of the water screen the proportion of infrared in the radiation from the lamp was reduced to a level considerably below that in sunlight. The visible portion of the spectrum of the tungsten filament lamp is deficient in the shorter wave lengths, as compared with sunlight. As regards intensity, values of 2,000 to 4,000 foot-candles were obtained immediately below the water screen, except at the outer edges near the walls of the compartment. The intensity decreased, of course, with increasing distance below the screen. The plants were grown in ordinary potting soil in wooden boxes. The containers were so adjusted as to bring the tops of the plants reasonably near the bottom of the water screen. Observations on the test plants were planned to show the effect of the different light treatments (1) on time of flowering and (2) on the nutrition and growth of the plants, as measured by appearance, height attained, and fresh and dry weights at the end of the test.

## EXPERIMENTAL DATA

### EXPERIMENTS WITH SUNLIGHT

In the first series of experiments, early, medium, and late varieties of soybeans and *Cosmos sulphureus* L., *Perilla frutescens* (L.) Britton, and *Impatiens balsamina* L. (garden balsam) were exposed alternately to a 10-hour day and to the full length of day during the latter part of June, July, and August. The control plants were exposed daily to the full length of day. Mandarin, the early variety of soybeans used, is relatively indifferent to seasonal changes in day length in the latitude of Washington. The three other varieties of soybeans and *Cosmos* and *Perilla* are to be classed as short-day plants, while garden balsam is a long-day type. Table 1 shows the results of the experiment with respect to date of first flowering and height attained by the plants at flowering.

In the next experiment, representative short-day and long-day plants were darkened throughout the daylight period and exposed to full-day illumination on alternate days. The controls were exposed to the full illumination period each day. Additional treatments included daily darkening of the plants from 10 a. m. to 3 p. m. or 10 a. m. to 2 p. m. and exposure to day lengths of 5, 8, 10, and 12 hours. The short-day plants used were Biloxi soybeans, *Tithonia tubaeformis* (Mill.) Blake, *Helianthus angustifolius* L., *Cosmos bipinnatus* Cav., and *Perilla frutescens* (L.) Britton. The long-day group



was represented by *Monarda didyma* L. and *Steironema ciliatum* (L.) Raf. The effects of the several treatments on date of first flowering and on average height of the plants are shown in Table 2.

TABLE 1.—Time of flowering and growth of plants of the short-day, indifferent, and long-day types exposed on alternate days during the summer months to full-day and to 10-hour day illumination as compared with daily full-day illumination

Type and species or variety	Treatment	First day of test	First flowering	Average height at flowering
				Inches
Short-day type:				
Peking soybeans.....	Full-day and 10-hour day alternations	June 29	July 21	6
Control.....	Full day daily	do	Aug. 10	12
Toyko soybeans.....	Full-day and 10-hour day alternations	do	Aug. 2	13
Control.....	Full day daily	do	Aug. 17	21
Biloxi soybeans.....	Full-day and 10-hour day alternations	do	Aug. 10	15
Control.....	Full day daily	do	Sept. 9	28
Cosmos sulphureus.....	Full-day and 10-hour day alternations	June 23	Aug. 30	38
Control.....	Full day daily	do	Oct. 15	
Perilla frutescens.....	Full-day and 10-hour day alternations	June 17	Aug. 11	21
Control.....	Full day daily	do	Sept. 9	30
Indifferent type:				
Mandarin soybeans.....	Full-day and 10-hour day alternations	June 29	July 23	7
Control.....	Full day daily	do	July 28	9
Long-day type:				
Impatiens balsamina.....	Full-day and 10-hour day alternations	June 17	July 12	9
Control.....	Full day daily	do	July 10	7

TABLE 2.—Effects of complete darkening on alternate days and of daily midday darkening on time of flowering and growth of short-day and long-day plants, as compared with effects of exposure to the full day and to various short days

Type and species or variety	Duration of daily illumination	First day of test	First flowering	Height of plants	
				Measured on -	Average
					Inches
Short-day type:					
<i>Tithonia tubaeformis</i> .....	5 hours.....	June 19		Aug. 2	32
Do.....	8 hours.....	do.....	July 25	do.....	33
Do.....	10 hours.....	do.....	do.....	do.....	38
Do.....	12 hours.....	do.....	Aug. 22	do.....	33
Do.....	Darkened on alternate days.....	do.....	Sept. 8	do.....	32
Do.....	Darkened 10 a. m.-3 p. m.....	do.....	Nov. 10	do.....	33
Control.....	Full day.....	do.....	do.....	do.....	15
<i>Helianthus angustifolius</i> .....	5 hours.....	June 12	Aug. 1	do.....	22
Do.....	8 hours.....	do.....	July 18	do.....	23
Do.....	10 hours.....	do.....	July 24	do.....	27
Do.....	Darkened on alternate days.....	do.....	Aug. 14	do.....	19
Do.....	Darkened 10 a. m.-3 p. m.....	do.....	Oct. 9	do.....	13
Control.....	Full day.....	do.....	Oct. 7	do.....	16.5
<i>Perilla frutescens</i> .....	10 hours.....	June 20	July 24	July 24	15
Do.....	Darkened on alternate days.....	June 9	July 17	July 17	14.5
Do.....	Darkened 10 a. m.-3 p. m.....	do.....	Sept. 1	do.....	
Control.....	Full day.....	do.....	Sept. 2	do.....	
Giant Crimson Cosmos.....	Darkened on alternate days.....	do.....	Aug. 4	Aug. 4	18
Do.....	Darkened 10 a. m.-3 p. m.....	do.....	Oct. 7	Oct. 7	72
Control.....	Full day.....	do.....	Sept. 28	Sept. 28	84
Biloxi soybeans.....	Darkened on alternate days.....	June 8	Aug. 3	Aug. 3	12
Do.....	Darkened 10 a. m.-3 p. m.....	do.....	Sept. 18	Sept. 18	8
Control.....	Full day.....	do.....	Sept. 11	Sept. 11	35
Long-day type:					
<i>Monarda didyma</i> .....	10 hours.....	Mar. 9		June 21	15
Do.....	Darkened 10 a. m.-2 p. m.....	do.....	June 21	do.....	26
Control.....	Full day.....	do.....	June 15	June 15	36
<i>Steironema ciliatum</i> .....	10 hours.....	Mar. 25			
Do.....	Darkened 10 a. m.-2 p. m.....	do.....	June 20	June 20	35
Control.....	Full day.....	do.....	June 29	June 29	28

In order to obtain an indication of the effects of further shortening the light-and-darkness alternations, the group of plants listed in Table

1 was darkened each day from 10 a. m. to noon and again from 2 to 4 p. m. during the summer and early fall. The controls were exposed to the full day length. The date of first flowering and average height of plants are shown in Table 3.

TABLE 3.—*Time of flowering and growth of plants of the short-day, indifferent, and long-day types darkened from 10 a. m. till noon and again from 2 to 4 p. m. each day during the summer months, as compared with full-day illumination*

Type and species or variety	Treatment	First day of test	First flowering	Average height at flowering
				<i>Inches</i>
<b>Short-day type:</b>				
Peking soybeans.....	Darkened twice a day.....	June 29.....	Aug. 10.....	9
Control.....	Full-day illumination.....	do.....	do.....	12
Tokyo soybeans.....	Darkened twice a day.....	do.....	Aug. 30.....	22
Control.....	Full-day illumination.....	do.....	Aug. 17.....	21
Biloxi soybeans.....	Darkened twice a day.....	do.....	Sept. 23.....	11
Control.....	Full-day illumination.....	do.....	Sept. 9.....	28
Cosmos sulphureus.....	Darkened twice a day.....	June 21.....	Oct. 11.....	24
Control.....	Full-day illumination.....	do.....	Oct. 20.....	51
Perilla frutescens.....	Darkened twice a day.....	June 30.....	do.....	17
Control.....	Full-day illumination.....	do.....	Oct. 16.....	25
<b>Indifferent type</b>				
Mandarin soybeans.....	Darkened twice a day.....	June 29.....	July 29.....	8
Control.....	Full-day illumination.....	do.....	July 28.....	9
<b>Long-day type:</b>				
Impatiens balsamina.....	Darkened twice a day.....	June 17.....	July 10.....	4
Control.....	Full-day illumination.....	do.....	do.....	9

#### EXPERIMENTS WITH ARTIFICIAL LIGHT

In the experiments with artificial light, a number of separate tests were first conducted in which all light treatments involved the same number of hours of total illumination daily, namely, 12 hours of light in the 24-hour cycle. Thus the total number of hours of illumination was the same as would be received by plants exposed to a 12-hour day. The first experiment in this group involved alternations of light and darkness of 6 hours in some instances and 4 hours in others, as compared with a 12-hour alternation. The long-day plant, *Rudbeckia bicolor* Nutt., and the Peking, Biloxi, and Mandarin varieties of soybeans were employed. The results are summarized in Table 4.

TABLE 4.—*Time of flowering and growth of plants exposed to 6-hour and 4-hour alternations of artificial light and darkness, as compared with 12-hour alternations*

Type and species or variety	Length of alternation	Duration of test	Time required for flowering	Average height at flowering
<b>Long-day type:</b>				
<i>Rudbeckia bicolor</i> .....	6	51	34	33
Control.....	12	51	29	33
<b>Indifferent type</b>				
Mandarin soybeans.....	6	51	31	45
Control.....	12	51	29	25
Mandarin soybeans.....	4	37	35	30
Control.....	12	37	21	24
<b>Short-day type:</b>				
Peking soybeans.....	6	51	(*)	42
Control.....	12	51	29	29
Peking soybeans.....	4	37	(*)	30
Control.....	12	37	21	20.5
Biloxi soybeans.....	6	51	(*)	40
Control.....	12	51	43	51

\* Had not flowered at termination of experiment.

The next step in the investigations was the use of much shorter alternations of light and darkness, retaining the 12-hour alternation as a control. The three light intervals employed in addition to the control interval were 1 hour, 1 minute, and 15 seconds, respectively. In some of the tests continuous illumination also was used for the sake of comparison. In these experiments soybeans, *Rudbeckia*, *Perilla*, and *Coleus* were used. In some instances the green and the dry weights of the plants were obtained at the end of the test. Young seedlings of soybeans, *Rudbeckia*, and *Perilla* were employed, while for the *Coleus* cuttings that had already made considerable growth were used. This *Coleus* is a short-day type, flowering in winter. The results of the test are shown in Table 5. Under the shorter light intervals the soybeans presented a pale, etiolated appearance, and the stems were of a spindling, stringy type of growth, particularly under the 1-minute intervals. In *Perilla* the leaves showed the deepest purple color under continuous illumination. Under the 12-hour illumination the leaves were almost pure green in color, as is usually the case when the plants are in the flowering condition. The 1-hour light period gave larger, finer leaves than the 1-minute or the 15-second intervals. In *Coleus* the 12-hour light period gave normally colored, large, healthy leaves, while continuous light and the shorter intervals resulted in decided etiolation and poor coloration. *Rudbeckia* suffered a reduction in growth under the shorter light alternations, including the 1-hour period; and under the 1-minute exposure the young seedlings were unable to survive.

For the purpose of obtaining a more detailed picture of the comparative effects on plants of alternations of various short periods of light and darkness a series of eight artificially lighted compartments was brought into use. The 12-hour light period was retained as a control, and the experimental periods of light and darkness used in the remaining seven compartments were 5 seconds, 15 seconds, 1 minute, 5 minutes, 15 minutes, 30 minutes, and 1 hour, respectively. In this group of experiments the short-day type of plant was represented by *Cosmos sulphureus*, the long-day type by *Rudbeckia bicolor*, *Delphinium ajacis* L., *Althaea rosea* Cav., and *Beta vulgaris* L., while of two additional species that were included, *Fagopyrum vulgare* Hill and *Ipomoea batatas* (L.) Poir., the former may be classed as belonging to the indifferent type, and little is known as yet concerning the proper classification of the latter. The results of the tests with respect to time of flowering, height attained, and the green and dry weights for the several species are summarized in Table 6.

TABLE 5.—Time of flowering and growth of plants exposed to equal alternations of artificial light and darkness of 15 seconds, 1 minute, and 1 hour, respectively, as compared with 12-hour alternations and continuous light

Type and species or variety	Length of alternations	Duration of test	First day of test	Time re-quired for flowering	Height of plants		Average green weight per plant			Average dry weight of 10 plants					
					Meas-ured on—	Average	Entire plant	Tops	Roots	Entire plants	Tops	Roots	Ratio of tops to roots		
Indifferent type: Mandarin soybeans	15 seconds	37	June 25	Days	July 17	Inches	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Ratio of tops to roots	
	1 minute	37	do	do	do	27	7.0	5.4	1.6	7.6	6.7	0.9	7.4	4.0	
	1 hour	37	do	do	do	27	4.2	2.8	1.4	3.7	3.2	5	6.4	3.1	
	12 hours	37	do	21	do	27	12.4	7.8	4.6	12.5	10.0	2.9	4.0	4.3	
	Control	37	do	do	do	27	12.4	7.8	4.6	11.8	8.9	2.9	3.1	4.3	
	15 seconds	37	do	do	do	27	12.4	7.8	4.6	14.7	11.1	7.8	4.3	5.6	
	1 minute	37	do	do	do	27	12.4	7.8	4.6	14.7	11.1	7.8	4.3	5.6	
	1 hour	37	do	do	do	27	12.4	7.8	4.6	14.7	11.1	7.8	4.3	5.6	
	Control	37	do	do	do	27	12.4	7.8	4.6	14.7	11.1	7.8	4.3	5.6	
	12 hours	37	do	34	do	27	17.5	5.4	1.6	7.6	6.7	0.9	7.4	4.0	
Short-day type: Biloxi soybeans	15 seconds	20	May 18	Days	June 6	June 6	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Ratio of tops to roots	
	1 minute	20	do	do	do	do	7.0	5.4	1.6	7.6	6.7	0.9	7.4	4.0	
	1 hour	20	do	do	do	do	4.2	2.8	1.4	3.7	3.2	5	6.4	3.1	
	12 hours	20	do	do	do	do	12.4	7.8	4.6	12.5	10.0	2.9	4.0	4.3	
	Control	20	do	do	do	do	12.4	7.8	4.6	11.8	8.9	2.9	3.1	4.3	
	15 seconds	20	do	do	do	do	12.4	7.8	4.6	14.7	11.1	7.8	4.3	5.6	
	1 minute	20	do	do	do	do	12.4	7.8	4.6	14.7	11.1	7.8	4.3	5.6	
	1 hour	20	do	do	do	do	12.4	7.8	4.6	14.7	11.1	7.8	4.3	5.6	
	Control	20	do	do	do	do	12.4	7.8	4.6	14.7	11.1	7.8	4.3	5.6	
	12 hours	20	do	do	do	do	12.4	7.8	4.6	14.7	11.1	7.8	4.3	5.6	
Perilla frutescens	Continuous light	43	June 7	Days	July 20	26	31.2	23.3	7.7	41.0	33.2	7.8	4.3	5.6	
	15 seconds	43	do	do	do	21.5	21.9	17.8	4.1	26.3	22.0	4.3	5.6	4.0	
	1 minute	43	do	do	do	18.5	40.2	30.9	8.9	69.8	56.8	10.2	4.3	5.6	
	1 hour	43	do	do	do	37	38.7	43.8	21.1	80.9	69.8	10.2	4.3	5.6	
	12 hours	43	do	32	do	38	47.2	46.4	3.2	97.8	80.9	21.3	3.3	4.3	
	Continuous light	43	do	do	do	9	41.5	36.4	3.2	28.5	23.0	4.3	5.6	5.2	
	15 seconds	43	do	do	do	9.5	48.0	43.3	4.1	28.5	23.0	4.3	5.6	5.2	
	1 minute	43	do	do	do	10	48.0	43.3	4.1	28.5	23.0	4.3	5.6	5.2	
	1 hour	43	do	do	do	10	48.0	43.3	4.1	28.5	23.0	4.3	5.6	5.2	
	12 hours	43	do	do	do	17	68.2	59.0	15.0	50.8	45.5	5.3	8.6	6.4	
Long-day type: Rudbeckia bicolor	Continuous light	43	do	do	do	17	68.2	59.0	9.2	46.2	41.2	5.0	8.2	8.2	
	15 seconds	56	Mar 23	Days	May 18	20	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0
	1 minute	56	do	do	do	20	16.8	16.8	16.8	16.8	16.8	16.8	16.8	16.8	16.8
	1 hour	56	do	do	do	20	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0
	12 hours	56	do	do	do	40	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9
	Control	56	do	do	do	31	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9
	Continuous light	56	do	do	do	31	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9
	15 seconds	56	do	do	do	31	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9
	1 minute	56	do	do	do	31	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9
	1 hour	56	do	do	do	31	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9

TABLE 6.—Time of flowering and growth of plants exposed to equal alternations of artificial light and darkness of 5 seconds, 15 seconds, 1 minute, 15 minutes, 30 minutes, and 1 hour, respectively, as compared with 12-hour alternations

Type and species or variety	Length of alternations	Duration of test	First day of test	Time required for flowering	Height of plants		Average green weight per plant			Average dry weight of 10 plants				
					Measured on—	Average	Entire plant	Tops	Roots	Entire plants	Tops	Roots	Ratio of tops to roots	
Days		Nov. 1		Days		Dec. 10		Grams		Grams		Grams		
Short-day type:														
Cosmos sulphureus	5 seconds	42	do	do	do	do	31	16.0	14.3	1.2	14.3	13.0	1.3	40.0
Do	15 seconds	42	do	do	do	do	13	1.5	1.5	.1	1.5	.5	.1	14.0
Do	1 minute	42	do	do	do	do	7	1.9	.6	.1	.6	.5	.1	3.0
Do	5 minutes	42	do	do	do	do	8.5	1.1	1.0	.1	1.0	.9	.1	8.0
Do	15 minutes	42	do	do	do	do	18	4.0	3.7	.1	3.3	3.0	.3	40.0
Do	30 minutes	42	do	do	do	do	20	3.3	3.1	.2	2.7	2.4	.3	8.0
Do	1 hour	42	do	do	do	do	22	11.0	10.0	1.0	9.9	9.0	.9	10.0
Do	12 hours	42	do	do	do	do	45	70.1	65.3	4.8	47.8	43.5	4.0	11.0
Control														
Long-day type:														
Rudbeckia bicolor	5 seconds	42	do	40	do	do	30	53.5	49.6	3.9	56.7	53.0	3.7	14.5
Do	15 seconds	42	do	40	do	do	25	30.5	26.5	3.7	38.7	35.0	3.0	13.7
Do	1 minute	42	do	40	do	do	17	13.0	14.0	1.0	17.1	15.5	1.6	8.1
Do	5 minutes	42	do	36	do	do	24	19.2	15.1	1.1	23.3	21.4	2.0	14.5
Do	15 minutes	42	do	36	do	do	25	27.3	23.1	1.2	29.6	26.6	2.6	18.5
Do	30 minutes	42	do	40	do	do	25	17.9	16.7	1.2	19.7	18.1	1.6	10.2
Do	1 hour	42	do	40	do	do	22	29.4	27.5	1.9	30.2	27.5	2.7	9.8
Do	12 hours	42	do	40	do	do	40	43.1	40.1	3.0	46.4	43.0	3.0	12.1
Control														
Delphinium elatum	5 seconds	50	Dec. 13	37	Feb. 1	do	46	23.6	21.3	2.5	52.5	48.5	3.0	13.0
Do	15 seconds	50	do	37	do	do	40	20.0	17.5	2.2	44.8	41.8	2.0	13.0
Do	1 minute	50	do	41	do	do	33	19.0	17.5	1.5	43.8	40.8	3.0	7.0
Do	5 minutes	50	do	45	do	do	27	11.5	10.2	1.3	27.5	25.0	2.5	10.9
Do	15 minutes	50	do	43	do	do	33	18.3	16.5	1.8	38.0	33.5	3.5	14.2
Do	30 minutes	50	do	42	do	do	39	25.8	22.5	3.3	52.5	47.0	5.5	8.2
Do	1 hour	50	do	43	do	do	36	28.9	25.6	3.3	52.3	47.3	5.0	9.4
Do	12 hours	50	do	41	do	do	42	85.0	76.3	15.0	130.0	97.0	33.0	29.9
Control														
Althaea rosea	5 seconds	50	do	40	do	do	31	103.0	85.7	17.0	130.6	97.0	33.0	4.4
Do	15 seconds	50	do	40	do	do	30	76.3	76.3	16.4	93.6	75.3	17.3	4.4
Do	1 minute	50	do	40	do	do	15	33.0	29.0	4.0	34.3	28.0	6.3	4.4
Do	5 minutes	50	do	40	do	do	11.5	34.0	31.0	3.0	38.0	31.0	7.0	4.4
Do	15 minutes	50	do	40	do	do	20	65.7	58.0	7.7	68.3	57.7	10.6	4.4
Do	30 minutes	50	do	40	do	do	27	97.3	84.3	13.0	112.0	99.0	22.0	4.1
Do	1 hour	50	do	40	do	do	28	72.0	63.0	9.0	86.0	68.3	17.7	4.1
Do	12 hours	50	do	40	do	do	25	136.0	123.0	24.0	190.0	131.0	53.9	2.2
Control														
Beta vulgaris	5 seconds	75	Mar. 22	June 5	do	do	18	77.5	68.4	8.4	88.5	67.0	21.8	1.2
Do	15 seconds	75	do	do	do	do	15	29.5	16.6	13.2	35.3	19.1	16.2	1.2
Do	1 minute	75	do	do	do	do	16	49.8	32.6	17.2	48.9	29.0	19.7	1.4
Do	5 minutes	75	do	do	do	do	20	68.5	49.7	19.0	70.0	42.9	27.1	1.4
Do	15 minutes	75	do	do	do	do	20	6.4	3.6	2.8	3.7	3.5	.1	20.0

Do.	30 minutes.	73	do.	do.	do.	18.5	13.9	4.7	15.5	10.9	4.6	2.4
Do.	1 hour.	73	do.	do.	do.	41.0	25.4	15.6	44.0	23.4	20.6	1.1
Control	12 hours.	73	do.	do.	do.	68.0	31.1	36.9	94.6	33.2	61.4	.5
Indifferent type:												
Fagopyrum vulgare												
Do.	5 seconds.	34	Feb. 7.	24	Mar. 12.	45.2	44.2	1.0	42.4	40.7	1.7	23.9
Do.	15 seconds.	34	do.	34	do.	18.5	18.2	.3	14.8	14.3	.5	28.5
Do.	1 minute.	34	do.	23	do.	12.0	11.6	.4	8.8	8.2	.6	13.7
Do.	5 minutes.	34	do.	24	do.	20.0	19.5	.5	16.0	15.2	.8	19.0
Do.	15 minutes.	34	do.	29	do.	10.6	10.3	.3	7.9	7.6	.3	25.3
Do.	30 minutes.	34	do.	29	do.	16.5	15.1	.4	11.1	10.6	.5	21.2
Do.	1 hour.	34	do.	29	do.	20.6	19.7	.9	13.5	13.4	1.1	17.1
Control	12 hours.	34	do.	21	do.	do.	32.3	do.	31.1	28.4	1.7	17.3
Type undetermined:												
Ipomoea batatas												
Do.	5 seconds.	98	Feb. 3.	May 11.	do.	248.0	146.0	102.0	644.0	122.0	26.0	.23
Do.	15 seconds.	98	do.	do.	do.	201.0	140.0	61.0	434.0	124.0	16.0	.40
Do.	1 minute.	98	do.	do.	do.	150.0	135.0	21.0	136.0	112.0	13.0	4.7
Do.	5 minutes.	98	do.	do.	do.	105.0	92.5	12.5	76.5	69.5	7.0	9.9
Do.	15 minutes.	98	do.	do.	do.	15.0	12.5	5.3	20.5	16.0	4.5	3.6
Do.	30 minutes.	98	do.	do.	do.	32.0	27.0	5.0	37.0	32.0	5.0	6.4
Do.	1 hour.	98	do.	do.	do.	43.0	35.0	8.0	48.5	44.0	4.5	9.8
Control	12 hours.	98	do.	do.	do.	352.0	221.0	131.0	931.0	179.0	32.0	.24

<sup>a</sup> Length of longest leaves.

<sup>b</sup> Includes weights of "tubers," which are not included in the column "roots."

The *Rudbeckia* seedlings used were somewhat older than those employed in the previous experiment, and the flowering stage was reached under all the short alternations. Under the light periods ranging from 1 minute to 30 minutes the plants were chlorotic, but under all other light treatments they showed the normal dark-green color. In *Cosmos* the same general relations existed, the etiolation being most marked under the 1-minute, 5-minute, and 15-minute light intervals, and the normal color showing under the 5-second, 1-hour, and 12-hour intervals. *Delphinium* seemed to be most adversely affected by the 5-minute light interval, and under this period only one plant survived. First-year seedlings of *Athaea rosea* were used in the test, and none flowered or developed flowering stems although the leaves developed abnormally long petioles. The 5-second and 12-hour plants showed the darkest green color, the 15-second and 1-hour individuals being next, and the others showing a decidedly lighter, yellowish green color. Only one seedling survived under the 5-minute light interval. For the experiment with the table beet (*Beta vulgaris*) young seedlings of the Early Eclipse variety were used. A single plant under the 1-minute light interval flowered, and one plant developed a stem under the 15-second light period. The heights recorded in Table 6 refer to the lengths of the longest leaves. The smallest plants, with leaves of poorest color, were those under the 15-minute light interval. The buckwheat (*Fagopyrum vulgare*) showed most etiolation under the 15-minute light interval, with progressive improvement in the green color under both longer and shorter intervals. In the sweetpotato (*Ipomoea batatas*) the foliage was yellow under the 15-minute, 30-minute, and 1-hour intervals, and no tubers or thickened roots were formed under these exposures or under the 5-minute interval. The best development of tubers occurred under the 12-hour and the 5-second intervals.

In all the foregoing experiments with artificial light the total number of hours of light and darkness received daily by the plants was the same. In the following series of tests the same plants and equipment were used, but the intervals of light and darkness were of unequal value, although the complete cycles remained the same as in the preceding experiments. In the first group of tests the interval of light was just half that of darkness in each case. For example, instead of the previous alternation of 5 seconds of light and 5 seconds of darkness, the new cycle consisted of  $3\frac{1}{2}$  seconds of light and 6 $\frac{1}{2}$  seconds of darkness; and in place of 1 hour each of light and of darkness, the new cycle was composed of 40 minutes of light and 80 minutes of darkness. In all cases the total daily illumination was 8 hours. As a control, an 8-hour light period was employed. Biloxi soybeans, *Cosmos sulphureus*, and *Rudbeckia bicolor* were used for the tests. The soybeans did not long survive the unfavorable effects of the  $\frac{1}{2}$ -minute,  $3\frac{1}{2}$ -minute, and 10-minute light intervals, and the *Cosmos* soon perished under these intervals and also under the 10-second and 20-minute intervals, so that data on comparative weights of the plants could not be obtained. Under these intervals the heights of the soybeans and *Cosmos* recorded were those reached at the time of death, while in other cases the heights attained at the end of the experiment are reported. The results of the tests are summarized in Table 7. None of the soybeans presented a healthy appearance

except those exposed to the 8-hour light period, and Cosmos also showed unfavorable effects from the shorter light intervals. Rudbeckia showed a more or less normal green color under the  $3\frac{1}{2}$ -second, 10-second, and 8-hour light periods.

In the final series of experiments the durations of the intervals used in the preceding tests were reversed, so that in each complete cycle the light interval was double the darkness interval, and the total daily illumination was 16 hours in each case. In the first experiment Rudbeckia and Cosmos were used. In this experiment an installation for automatic control of temperature and relative humidity was employed. A current of conditioned air sufficiently rapid to insure substantially the same temperature and humidity in each compartment was forced through the light compartments. On four unusually warm days the midday temperatures reached 75° to 80° F. and on a single day 90°. At other times the temperature was held at  $71^{\circ} \pm 2^{\circ}$  and the relative humidity was maintained at  $53 \pm 2$  per cent. The weekly mean temperature ranged from 71° to 75° during the period of the test. In the early morning of the thirty-fifth day there was a breakdown in the control system that caused the temperature to rise above 100° for a few hours. It was therefore decided to discontinue the experiment. The Cosmos plants were so badly dried out by the excessive heat that satisfactory data on weights of the plants could not be obtained. The Rudbeckia, however, showed no sign of material injury.

The increased illumination produced obvious improvement in nutrition and growth under all treatments, as compared with the results in the preceding experiment; but the contrasts in condition of the plants under the different light treatments were more or less similar to those in the preceding experiment. The Rudbeckia plants had not flowered when the test was interrupted, but all had formed flower buds. None of the Cosmos plants showed flower buds. The results of the experiment are summarized in Table 8. A similar experiment with Mandarin and Peking soybeans is also shown in Table 8. In this case an effort was made to maintain the temperature at 77° F. and the relative humidity at 55 per cent so far as weather conditions would permit. As it happened, the weather was unseasonably warm for a considerable portion of the time so that the temperature was beyond control, and toward the end of the experiment it became necessary to cut off all artificial heat.



TABLE 7.—Time of flowering and growth of plants exposed to various short alternations of artificial light and darkness in a ratio of 1 to 2, as compared with alternations of 12 hours each of light and darkness or 8 hours of light and 16 hours of darkness

Type and species or variety	Length of alternations		Duration of test	Time required for flowering	Average height of plants	Average green weight per plant			Average dry weight of 10 plants			Ratio of tops to roots	
						Light		Darkness	Entire plant	Tops	Roots		Entire plants
	Grams	Inches				Grams	Grams						
Short-day type: Bicolor soybeans	3 1/2 seconds	6 1/2 seconds	41	Days	Inches	Grams	Grams	Grams	Grams	Grams	Grams	Grams	
	10 seconds	20 seconds	41		30								
	Do.	1 1/4 minutes	41		25								
	Do.	2 1/4 minutes	41		14								
	Do.	3 1/2 minutes	41		19								
	Do.	20 minutes	41		17.5								
	Do.	40 minutes	41		25								
	Do.	2 1/4 hours	41		30								
	Do.	12 hours	41		36								
	Control	3 1/2 seconds	6 1/2 seconds	39		13							
Cosmos sulphureus	10 seconds	20 seconds	39		5								
	Do.	3 1/2 minutes	39		6.5								
	Do.	20 minutes	39		7								
	Do.	40 minutes	39		13								
	Do.	2 1/4 hour	39		32								
	Do.	8 hours	39										
	Control	3 1/2 seconds	50		32	9.1	2	0.5	9.3	5	0.5	17.6	
	Do.	10 seconds	50		28.5	1.5	1.5	1.5	1.5	1.5	1.5	19.0	
	Do.	3 1/2 minutes	50		38	4.3	4.3	4.3	4.3	4.3	4.3	28.0	
	Do.	20 minutes	50		40	6	6	6	6	6	6	17.0	
Long-day type: Rudbeckia bicolor	10 seconds	20 seconds	50		21	4.5	4.5	4.5	4.5	4.5	4.5	18.0	
	Do.	3 1/2 minutes	50		42	6.4	6.4	6.4	6.4	6.4	6.4	11.5	
	Do.	20 minutes	50		45	7.4	7.4	7.4	7.4	7.4	7.4	14.8	
	Do.	40 minutes	50		49	9.8	9.8	9.8	9.8	9.8	9.8	14.8	
	Do.	2 1/4 hour	50		5	24.5	24.5	24.5	24.5	24.5	24.5	6.4	
	Do.	8 hours	50										
	Control	3 1/2 seconds	50		35	30.0	24.5	3.5	28.1	24.5	3.5	6.4	
	Do.	10 seconds	50		35	9.1	2	0.5	9.3	5	0.5	17.6	
	Do.	2 1/4 minutes	50		38	4.3	4.3	4.3	4.3	4.3	4.3	28.0	
	Do.	3 1/2 minutes	50		40	6	6	6	6	6	6	17.0	

TABLE 8.—Time of flowering and growth of plants exposed to various short alternations of artificial light and darkness in a ratio of 2 to 1, as compared with an alternation of 16 hours of light and 8 hours of darkness

Type and species or variety	Length of alternations		Duration of test	Time required for flowering	Average height of plants	Average green weight per plant			Average dry weight of 10 plants		
	Light	Darkness				Entire plant	Tops	Roots	Entire plants	Tops	Roots
Short-day type: Cosmos sulphureus	6½ seconds	3¼ seconds	36	Days	Inches	Grams	Grams	Grams	Grams	Grams	Grams
	20 seconds	10 seconds	36		25						
	1¼ minutes	¾ minute	36		14.5						
	6¾ minutes	3¾ minutes	36		9						
	20 minutes	10 minutes	36		18						
	40 minutes	20 minutes	36		22						
	1¼ hours	¾ hour	36		23						
	16 hours	8 hours	36			8.1	7.5	0.6	13.5	12.8	0.7
	Control	Control	36			10.4	9.6	0.8	12.7	11.9	0.8
	Peking soybeans	3½ seconds	42		50	15.3	14.3	1.0	20.9	19.9	1.0
	Do	10 seconds	42		50	10.2	9.1	1.1	13.7	12.9	0.8
	Do	2¼ minute	42		50	9.2	8.5	0.7	12.7	11.8	0.9
Do	3¼ minutes	42		50	8.2	7.6	0.6	11.7	11.1	0.6	
Do	20 minutes	42		50	11.6	10.7	0.9	16.2	15.1	1.1	
Do	40 minutes	42		42	12.4	11.6	0.8	15.1	14.0	1.1	
Do	1¼ hours	42									
Do	2¼ hour	42									
Do	8 hours	42			44						
Control	Control	42									
Long-day type: Rudbeckia bicolor	6½ seconds	3¼ seconds	36	• 35	25	23.2	22.0	1.2	21.4	19.9	1.5
	20 seconds	10 seconds	36	• 35	21	16.1	15.0	1.1	17.1	15.7	1.4
	1¼ minutes	¾ minute	36	• 35	20	18.1	16.8	1.3	14.4	12.9	1.5
	6¾ minutes	3¾ minutes	36	• 35	14.5	14.0	12.3	1.7	15.0	13.0	2.0
	20 minutes	10 minutes	36	• 35	18	16.3	14.7	1.6	16.6	14.7	1.9
	40 minutes	20 minutes	36	• 35	22	15.9	14.8	1.1	16.9	14.9	2.0
	1¼ hours	¾ hour	36	• 35	22	29.9	27.6	2.3	30.4	27.1	2.3
	16 hours	8 hours	36	• 35	23	25.4	23.7	1.7	18.9	17.0	1.9
	Control	Control	36								
	Nonfliferent type: Nandarin soybeans	3½ seconds	42		50	12.7	11.7	1.0	15.7	14.9	0.8
	Do	10 seconds	42		50	17.1	16.3	1.0	22.6	22.0	1.2
	Do	20 seconds	42		50	13.5	13.6	1.2	16.7	15.8	1.3
Do	¾ minute	42		50	13.4	13.4	1.0	16.7	15.7	1.9	
Do	3¾ minutes	42		50	13.4	13.4	1.0	16.7	15.7	1.9	
Do	20 minutes	42		50	12.6	11.9	1.0	16.9	16.1	0.8	
Do	40 minutes	42		46	15.4	14.4	1.0	20.3	19.6	0.9	
Do	1¼ hours	42		50	22.9	21.6	1.3	35.2	33.0	2.2	
Do	2¼ hour	42									
Do	8 hours	42									
Control	Control	42		32							

\* Flower buds were present but none had opened.

A summary of the thermographic records obtained in the light chambers during the experiments is given in Table 9. The data include the maximum and minimum temperatures recorded in each series; also the mean maximum, the mean minimum, and the approximate mean or average of the latter two values.

TABLE 9.—*Temperature in light-proof chambers used in experiments summarized in Tables 5 to 8*

Table No. and plant	Temperature of light chambers (° F.)				
	Maximum	Minimum	Mean maximum	Mean minimum	Mean of mean maximum and mean minimum
Table 5:					
Mandarin and Biloxi soybeans.....	88	64	82	71	76
Rudbeckia.....	99	60	88	72	80
Biloxi soybeans.....	84	65	80	67	74
Perilla and Coleus.....	86	66	84	70	77
Table 6:					
Rudbeckia and Cosmos.....	81	65	75	69	72
Delphinium and Althaea.....	77	64	72	68	70
Fagopyrum.....	79	65	74	69	72
Beta.....	80	60	73	67	70
Ipomoea.....	80	62	74	67	71
Table 7:					
Biloxi soybeans and Cosmos.....	85	70	83	76	80
Rudbeckia.....	87	61	78	71	75
Table 8:					
Rudbeckia and Cosmos.....	90	69	74	71	73
Mandarin and Peking soybeans.....	90	62	82	73	78

## DISCUSSION OF RESULTS

In nature, plants are normally exposed to a 24-hour cycle of day and night, there being, of course, only one period of light and one of darkness in the cycle. Under these circumstances the variation in relative length of day and night dependent upon latitude and season may profoundly affect the development of the plant. By excluding the daylight of early morning or late afternoon so as to shorten the daily light period, on the one hand, and by using artificial light to prolong the daily illumination, on the other hand, the distinctive effects of short days and long days on flowering and fruiting and other features of growth may be reproduced, under suitable conditions, in any latitude and at any season. Results of the experiments presented in this paper, however, indicate that different variations from the 24-hour cycle of light and darkness may produce radically different effects, even though the total number of hours of illumination is the same.

In previous experiments it has been shown that diminishing the number of hours of the midsummer daylight to which plants are continuously exposed hastens the flowering of short-day plants and retards that of long-day plants, whereas an equal period of darkening in the middle of the day, by which the short day is divided into two parts, has little or no effect on the time of flowering.

Table 1 summarizes the results of an experiment in which short-day and long-day plants were subjected on alternate days to a full day and to a 10-hour day of sunlight. These data show that under the conditions that prevailed the interposition of the long day did

not nullify the hastening effect of the short day on the flowering of the short-day plants, while it did very nearly prevent the retarding action on the long-day plants. Reduction in height attained was associated with early flowering under the long-day and short-day alternations.

When the 24-hour cycle of day and night was replaced by a 48-hour cycle (illumination on alternate days, see Table 2), consisting of about 15 hours of light and 33 hours of darkness, there was a pronounced hastening action on flowering in five typical short-day plants. The short-day effect, therefore, is still in evidence, although comparison with the results of 8, 10, and 12 hours of light daily shows that the hastening effect has been much reduced in *Tithonia* and *Helianthus*.

The present data, however, bear chiefly on the effects of cycles of light and darkness much shorter than the 24-hour cycle provided



FIGURE 1—Fringed loosestrife (*Steironema ciliatum* (L.) Raf.), a long-day plant, exposed to the full day length of late spring and early summer and to regulated daily light periods, beginning March 25. The illumination conditions were: A, Full day; B, darkened 10 a. m. to 2 p. m.; C, 12-hour day; D, 10-hour day; E, 8-hour day; F, 5-hour day. Midday darkening (B) failed to suppress flowering, the usual effect of the short day (C-F)

by nature. The results in Table 2 bring out the remarkable fact that darkening during the middle of the day for as long as 5 hours has either no effect at all or only a slight effect on the time of flowering as compared with the full day length of summer. This is in sharp contrast with 8, 10, or 12 hours of uninterrupted daily illumination. In earlier experiments much the same results were obtained by a 2-hour midday darkening. All short-day plants studied have failed to show response to midday darkening with respect to flowering; that is, they have behaved about the same as when exposed to the full day length. The majority though not all of the long-day plants tested also have failed to respond to the midday darkening.

The ineffectiveness of midday darkening in delaying elongation of flowering stems in *Steironema* is shown in Figure 1. It will be observed that the effect of this treatment was to break up the normal 24-hour cycle, for in each 24-hour period the plants were subjected to two intervals of light and two intervals of darkness. The Stei-

ronema plants exposed to the full day length, beginning March 25, began flowering June 29; those darkened in the middle of the day began flowering nine days earlier; all other treatments suppressed flowering. Midday darkening, even though prolonged, as a rule does not produce the usual effects of a short day on either the long-day or the short-day type of plant.

Further evidence that relatively short cycles of light and darkness tend to produce effects similar to those of a long day or of continuous light in that they favor flowering in long-day plants but not in short-day plants is furnished by the results shown in Table 3. Three daily light periods, aggregating about 10 hours in duration, failed to materially change the date of flowering in the long-day or indifferent types, and in the short-day plants either failed to change or actually delayed the date of flowering, as compared with the effect of the full summer-day length. With one exception, the darkening had the effect of reducing the heights of the plants.

#### EFFECTS ON FLOWERING

As regards the normal 24-hour cycle, an even division into 12 hours of daylight and 12 hours of darkness permits of eventual flowering and fruiting in the majority of plants of both the long-day and short-day types. As a rule, however, the 12-hour day is above the optimum for flowering in the short-day group and below the optimum for the long-day group. In studying with artificial illumination the effects of relatively short cycles involving an even distribution of light and darkness, the 12-hour day naturally was employed as the control. In the long-day plant *Rudbeckia*, a 6-hour alternation of light and darkness somewhat hastened the appearance of blossoms. (Table 4.) In the short-day varieties of soybeans, flowering was decidedly delayed by 6-hour and 4-hour alternations. The effects on height attained by the plants were somewhat variable.

Results obtained with alternations of light and darkness of 1 hour or less soon showed that a distinction must be drawn between effects on reproduction and those on growth and general nutrition of the plant. In the experiments with alternations of light and darkness of 1 hour, 1 minute, and 15 seconds (Table 5) and those including alternations of 1 hour, 30 minutes, 15 minutes, 5 minutes, 1 minute, 15 seconds, and 5 seconds (Table 6), it will be observed that flowering did not take place in the short-day plants—soybeans, *Perilla*, *Coleus* and *Cosmos*—under any of these exposures, the effect being that of a long day or continuous light. Under a 12-hour day all flowered except *Coleus* and *Cosmos*, and these had formed flower buds when the experiment was stopped. Of the long-day plants used, it is evident that *Rudbeckia* flowered much earlier under all the short alternations than under the 12-hour day, and about the same time as under continuous illumination. *Delphinium*, a less pronounced long-day plant, flowered about the same time under all of the light periods. The first-year plants of *Althaea*, another long-day type, did not flower under any of the alternations. The beet (*Beta*) is a long-day plant and when exposed to a long day and a cool temperature may behave as an annual. Under the warm temperature of the present experiment a single plant flowered with the

1-minute alternations, and a single seed stalk was formed with the 15-second alternations, although it did not flower. These results show a tendency of the plants under the short alternations to behave as when exposed to a long day. Buckwheat (*Fagopyrum*), which is relatively indifferent to changes in day length with respect to flowering, apparently was slightly delayed in flowering by the short alternations. The sweetpotato (*Ipomoea*) is ordinarily a nonflowering type of plant at the higher latitudes, and failed to blossom in the present experiment.

With the cycles remaining the same as in the preceding tests but with the light periods having one-half the value of the periods of darkness (Table 7), *Rudbeckia* still flowered under all the short alternations; but flowering was progressively delayed with increase in the length of the alternations. Biloxi soybeans again failed to flower under the short alternations. After 39 days *Cosmos* was showing flower buds only under the 8-hour day. Therefore, even with a total of only 8 hours of illumination daily, the effect on flowering of the short alternations is essentially that of a long day or continuous light. Finally, with the same cycles as before but with the light periods double the periods of darkness (Table 8), *Rudbeckia* readily developed flower buds. At the end of 36 days *Cosmos* had formed no flower buds, and neither the Peking nor Mandarin soybeans had flowered. The Peking variety of soybeans is a short-day type, while the Mandarin behaves as such only in regions having a summer day length of more than 17 hours. Here, again, with total daily illumination of 16 hours, the effects of the short alternations are those of a long day or continuous light on both the long-day and the short-day type of plant.

#### EFFECTS ON GROWTH AND GENERAL NUTRITION

In the experiments with short alternations of light and darkness of equal duration it was quickly observed that pathological symptoms are apt to develop and that the severity of the symptoms depends on the particular alternations employed. In soybeans it was found that the leaves are most severely affected by the 1-minute intervals of light and darkness. The leaves may be much reduced in size, assume a pale yellowish green color, and soon develop numerous areas of dead tissue. (Fig. 2.) Similar though less pronounced effects were produced by other alternations, ranging from 30 minutes to 15 seconds, but the leaves developed normally when the alternations of light and darkness were reduced to 5 seconds. Other plants also showed more or less severe pathological symptoms in the leaves when exposed to the light intervals of 1 minute or to those ranging from 15 seconds to 15 minutes, although the symptoms were not always so clearly defined as in soybeans. In some instances young seedlings of *Rudbeckia* and other plants were unable to survive under the 1-minute light intervals. Destruction of chlorophyll seems to be an important feature in the unfavorable effects of these particular alternations of light and darkness. With change in the length of the light interval in either direction there was definite improvement in the appearance of the foliage leaves. With a decrease to 5 seconds or an increase to 30 minutes or an hour in the duration of the intervals, the plants usually became normal in color and gen-

eral appearance, except sweetpotatoes, which were still chlorotic under the 1-hour intervals.

Attenuation was another outstanding feature in the unfavorable effects of the intermediate intervals of light and darkness. In soybeans the stems were not necessarily shortened, but they were weak and much reduced in size, thus tending to become viny. In most other species, also, the stems seemed to be abnormally small in comparison with the height, as if there were a stimulus toward stem elongation out of proportion to the basal nutrition of the plant.

The results shown in Tables 5 and 6 make it clear that, except in soybeans, the heights attained by the plants usually were reduced by the alternations ranging from 15 seconds to 30 minutes or an hour.

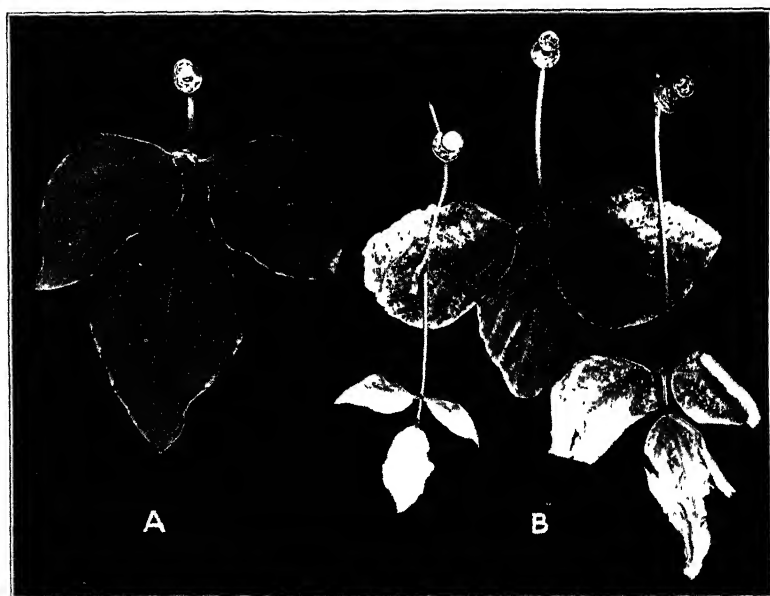


FIGURE 2—Leaves of soybeans (*Soja max* (L.) Piper) grown with artificial illumination: A, Leaves of plants exposed to 12-hour alternations of light and darkness, normal in color and general development, B, leaves of plants exposed to 1-minute intervals of light and darkness, markedly chlorotic and much reduced in size, containing numerous spots of dead tissue, and showing a tendency to die prematurely

It is quite clear, also, that the 5-second alternation generally gave increases in height almost or quite equal to those produced by the relatively long alternations used as controls. In the long-day plant *Rudbeckia*, the 12-hour day retarded elongation of the flowering stem, as would be expected, and some indication of this effect persisted in the 1-hour alternation. The comparative effects of the different alternations on the height attained, as well as on flowering and degree of attenuation, in *Cosmos* and *Delphinium* are well shown in Figures 3 and 4. It is seen that in the former there is a progressive decrease in stem elongation and in size or mass of the plants from the 12-hour alternations down to the 1-minute periods of light and darkness, and a corresponding increase in elongation and stockiness as the periods are further shortened to five seconds. In *Delphinium* the same relations hold, except that the maximum depressing effects are produced

with the 5-minute intervals and there is even greater improvement in growth with the 5-second alternations. In the short-day plant *Cosmos*, flower buds formed only under the 12-hour intervals of light and darkness; whereas in the long-day type *Delphinium*, flowering occurred with equal readiness under all alternations employed.

Total growth, as measured by green weights and oven-dried weights of the entire plants, shows much the same effects from the different alternations of light and darkness as does the height attained by the plants. In nearly all cases maximum depressing effects on green and dry weights of the plants are associated with maximum depression in height. In practically every instance the minimum green weight

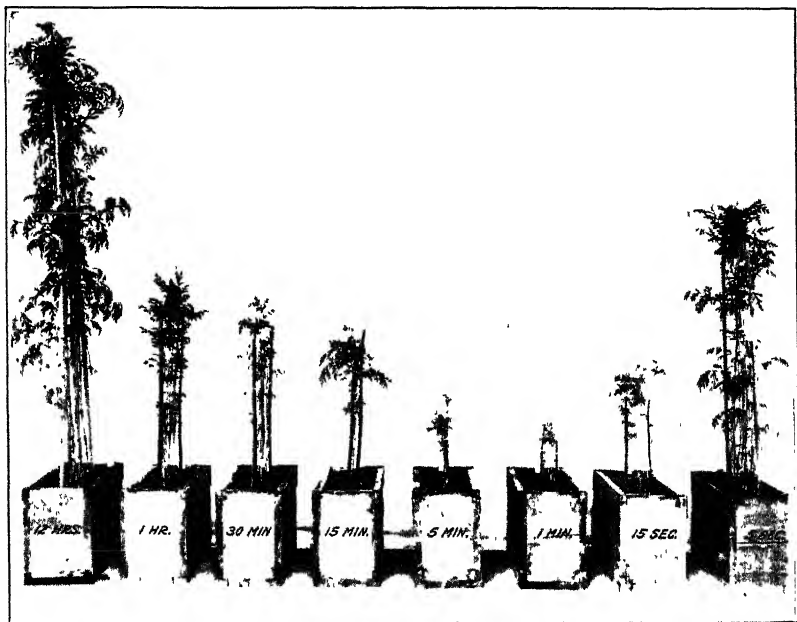


FIGURE 3.—Yellow cosmos (*Cosmos sulphureus* Cav.), a short-day plant, grown with equal alternations of light and darkness ranging from 12 hours to 5 seconds. With decrease in the intervals of light and darkness there is progressive decrease in height, size, and weight of the plants (see Table 6) and increase in etiolation and attenuation till the 1-minute intervals are reached. Further shortening of alternations causes marked improvement in growth and appearance of the plants. All intervals from 1 hour downward are almost equally unfavorable for flowering

is associated with minimum dry weight. The indicated values for water content of the green plants can be regarded as only approximations and do not show any very definite contrasts under the different alternations, except that in several instances the water content seems to be relatively high under the 12-hour alternations. Data were collected on the relative weights of tops and roots under the different treatments, and values for the ratio of tops to roots are included in the tables. The values for weights of roots are not accurate, however, because of difficulties in recovering all roots from the soil, and no great importance can be attached to the results. In the case of *Ipomoea* no tubers were formed under the alternations ranging from five minutes to one hour. To obtain some indication as to whether failure in absorption of soil nutrients could be responsible for the unfavorable



results with the intermediate short alternations, determinations of total ash in the tops of *Delphinium* and *Fagopyrum* (Table 6) were made by C. W. Bacon, of the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry. With alternating periods of light and darkness of 12 hours, 5 minutes, and 5 seconds, the corresponding percentages of total ash in *Delphinium* were 12.15, 13.64, and 10.49, respectively; in *Fagopyrum*, alternations of 12 hours, 15 minutes, and 5 seconds gave percentages of 14.97, 18.80, and 13.77, respectively. These data give no indication of deficiency in ash constituents under the harmful alternations of light and darkness.

When the light periods were reduced to one-half the periods of darkness, without change in duration of the total cycles, the unfavor-

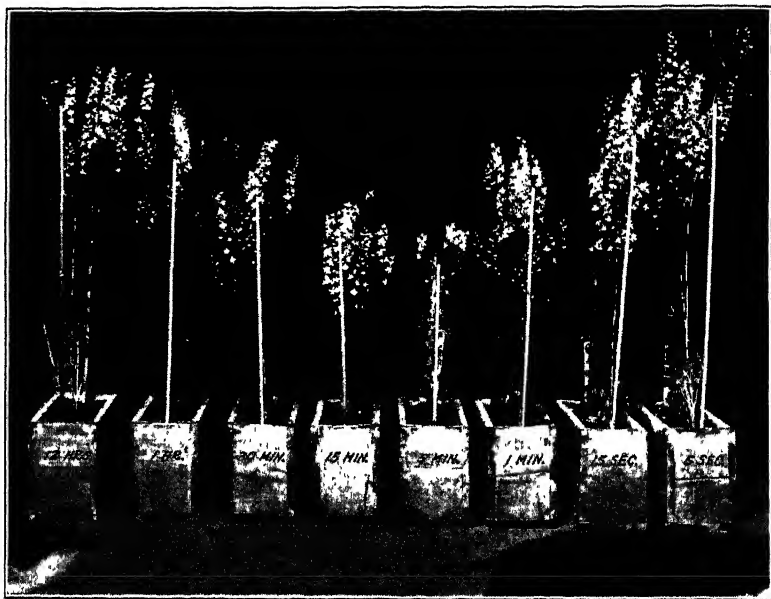


FIGURE 4.—Rocket larkspur (*Delphinium ajacis* L.), a long-day plant, grown with equal alternations of light and darkness ranging from 12 hours to 5 seconds. The comparative effects of the different alternations on nutrition and growth are about the same for long-day as for short-day plants. (Fig. 3.) As regards flowering, however, it is apparent that none of the shorter alternations show a retarding action, whereas all have this effect on short-day plants

able effects on growth of the cycles ranging from 30 seconds to 1 hour were accentuated. (Table 7.) *Cosmos* soon died when exposed to these alternations, and soybeans were able to survive only under the 30-second and 1-hour cycles, so that satisfactory data on green and dry weights could not be obtained. *Rudbeckia* survived under all treatments, and the effects on growth of reducing the light periods to one-half the periods of darkness, as compared with equal alternations of light and darkness in the corresponding cycles, are indicated in Figures 5 and 6. The relative effects of the different cycles on growth are approximately the same in the two cases, but it is obvious that reduction in the ratio of light to darkness in each cycle has greatly reduced the vigor and the amount of growth. This is clearly shown also in the green and dry weights recorded in Tables 6 and 7. The

retarding action on stem elongation of the 12-hour alternation decreases progressively in the next three shorter alternations (fig. 5), and the retarding action of the 8-hour light period decreases in the next two shorter alternations (fig. 6).

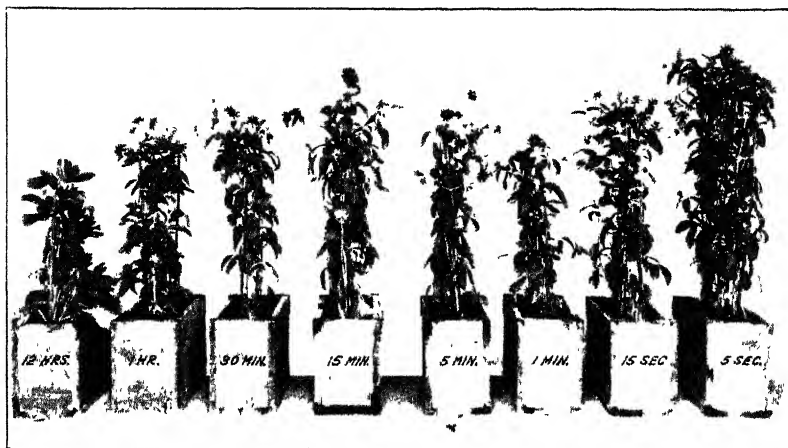


FIGURE 5.—Pinewoods coneflower (*Rudbeckia bicolor* Nutt.), a more pronounced long-day plant than Delphinium (fig. 4), grown with equal alternations of light and darkness ranging from 12 hours to 5 seconds. The relative effects of the different alternations on growth and on flowering resemble those in Delphinium, except that the 12-hour light period retards stem elongation and flowering, and these effects still persist in decreasing degree with the 1-hour and the 30-minute periods. In this case maximum growth was obtained with the 5-second alternations. (Table 6)

When the light period was increased to twice the period of darkness in each of the cycles, there was decided improvement in the vigor and general nutrition of the plants. (Table 8.) In *Rudbeckia* and

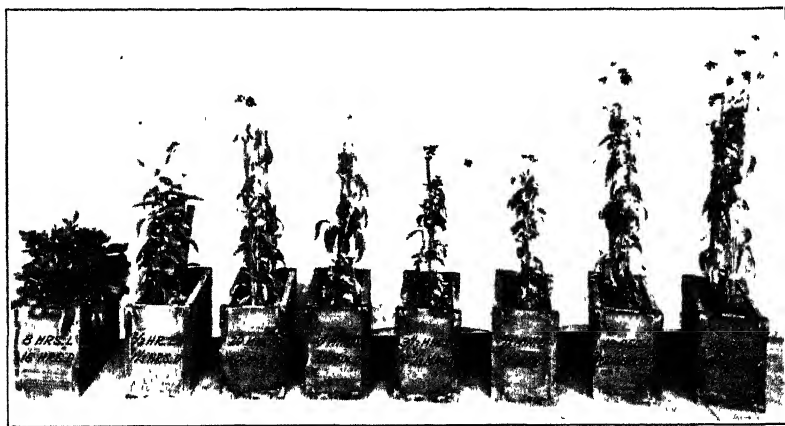


FIGURE 6.—Pinewoods coneflower grown with the same total cycles of light and darkness as those applied to the plants shown in Figure 5, but with the light periods in each cycle only one-half as long as the periods of darkness. Thus the plants in all cases received a total of 8 hours of light daily instead of 12 hours. There was no fundamental change in relative effects of the different cycles on growth and flowering, but comparison with Figure 5 shows there was a decided reduction in growth of the plants in all cases.

Cosmos the same differential effects of the shorter alternations previously noted were still in evidence with respect to both height and weight in the former and height in the latter plants. With soybeans,

however, the increased ratio of light to darkness seemed largely to overcome the adverse effects of the alternations ranging from 15 seconds to 30 minutes. The heights attained by *Rudbeckia* and *Cosmos* are comparable to those produced with an even ratio of light and darkness, except under the 16-hour as compared with the 12-hour light period. In *Rudbeckia* the increase in the ratio of light to darkness failed to increase the weights of the plants.

The results presented in this paper relate primarily to effects of relatively short alternations of light and darkness ranging from 6 hours to 5 seconds, with a 12-hour alternation of light and darkness as the principal basis for comparison. As already pointed out, the 12-hour alternation, which supplies the only equal distribution of light and darkness occurring in the normal 24-hour cycle, is not optimum for flowering in most plants of either the long-day or the short-day type. A light-and-darkness ratio of 5:7, as supplied in a 10-hour day, or a ratio of 2:1, as in an 8-hour day, is more likely to be optimum for most short-day plants; and the reverse ratios of 7:5 and 2:1, as in day lengths of 14 and 16 hours, respectively, are more nearly optimum for typical long-day plants. It will be of considerable interest in this connection to study the effects of 10-hour and 8-hour alternations having equal periods of light and darkness, and other alternations between those of 12 hours and 6 hours with both equal and unequal periods of light and darkness, which do not occur in the natural 24-hour cycle. It is hoped that these studies can be carried out with rather rigid control of temperature and other environmental factors.

#### SUMMARY

Breaking the continuity of the daily illumination period of plants by darkening them in the middle of the day for periods of 1 or 2 hours to as long as 5 hours may materially affect the general nutrition and amount of growth, but as a rule it fails to influence reproductive activities to a degree at all comparable with that produced by excluding the early morning or late afternoon light of the long summer days. This applies to all short-day plants that have been studied and to a majority of long-day plants, and means that reducing the hours of daily illumination by thus breaking the continuity of the illumination is not generally effective in inducing reproductive activity in short-day plants or in interfering with initiation of the process in long-day plants. The effects are essentially those of the long day.

Further breaking up of the daylight period by darkening the plants from 10 a. m. to noon and from 2 to 4 p. m. seemed to accentuate the lack of effectiveness of this method of reducing the hours of illumination in bringing about the characteristic effects of a short-day length. In some short-day plants flowering was actually delayed as compared with results under the full day of summer.

When the test plants were completely darkened on alternate days during the summer months, thus substituting a 48-hour cycle of light and darkness for the normal 24-hour cycle and furnishing about 15 hours of light and 33 hours of darkness in the cycle (corresponding to  $7\frac{1}{2}$  hours of light daily), the effects were those of a short day, although these effects were much weaker than those of a normal 8-hour or 10-hour day.

Exposing a group of short-day plants alternately to a 10-hour day and to the full day length of summer produced an effect intermediate between the effects of a long day and a short day as regards initiation of flowering. *Impatiens balsamina* (garden balsam), the only long-day plant tested, behaved as when exposed daily to a long day.

Following the experiments in which sunlight was the source of illumination, a number of species were grown under a series of different, relatively short alternations of light and darkness ranging from six hours to five seconds, the plants being illuminated with electric light of high intensity. The plants were grown at approximately outdoor summer temperatures in small light-proof chambers fitted with 1,000-watt Mazda lamps and provided with forced ventilation. In the major series of tests the duration of the light intervals was the same as that of the intervals of darkness in all the alternations. The differential effects of the several alternations on general nutrition and growth were in striking contrast with the comparatively uniform action on initiation of flowering.

As the equal intervals of light and darkness, beginning with 6 hours, were progressively shortened, there was increasing evidence of malnutrition and retardation in growth, which in several instances culminated in the 1-minute intervals. With further shortening of the alternations below the point at which maximum injurious action occurred, there was pronounced improvement in the nutrition and growth of the plants, so that the 5-second alternations often gave about as good results as the 12-hour controls. The injurious effects of the intermediate alternations included apparent destruction of chlorophyll, general etiolation, localized dying of the leaf tissue, reduction in leaf development, attenuation, and decrease in stem elongation and in production of dry matter. The effects on short-day plants and long-day plants seemed to be much the same.

When, with the same total cycles of light and darkness, the light intervals were reduced to one-half the intervals of darkness, the unfavorable effects of the intermediate alternations were intensified to such an extent that most of the plants soon died. On the other hand, increase of the light interval to twice the interval of darkness in each of the cycles had the effect of improving the general appearance of the plants exposed to the intermediate alternations, and in some cases seemed largely to overcome the retarding action of these alternations on growth.

As regards action on flowering, the effects in any particular case seemed to depend mainly on whether the plant belonged to the long-day or short-day type rather than on the duration of the alternations. In general, all the alternations, from 6 hours downward, were found to favor flowering in long-day plants but were unfavorable for flowering in short-day plants. The effects on flowering, therefore, are much the same as those resulting from midday darkening with natural illumination. The plants behave essentially as if exposed to a long day or continuous illumination.



# GERMICIDAL EFFICIENCY OF ORTHOPHENYLPHENOL AGAINST MYCOBACTERIUM TUBERCULOSIS<sup>1</sup>

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## INTRODUCTION

Of the commonly used disinfectants, saponified cresol solution, which consists essentially of phenols dissolved in soap solution, is the only one which has been found to be satisfactorily effective against *Mycobacterium tuberculosis*. This product is readily available on the market and is low in cost. Unfortunately, however, it has a strong odor which is likely to be absorbed by food products exposed to it. This fact limits its usefulness in the work of the Bureau of Animal Industry, practically prevents its employment in packing houses, and gives rise to complaints of tainted milk when it is employed in dairy barns and similar places. For some time past, therefore, a search has been made in the laboratories of the Biochemic Division for a disinfectant that would be efficient but free from objectionable odor. Among the substances studied was orthophenylphenol, one of the higher phenols which is practically odorless when pure. Like many of the higher phenols it is only slightly soluble in water but is readily soluble in the presence of certain soaps, thus affording solutions somewhat similar to saponified cresol solution. It may also be dissolved by the aid of alkalies.

The work, the results of which are reported in this paper, was carried out to determine the germicidal efficiency against *Mycobacterium tuberculosis*<sup>2</sup> of solutions of orthophenylphenol prepared by the aid of soap or alkalies.

## EXPERIMENTAL PROCEDURE AND RESULTS

The test organism used in all these experiments was a strain of *Mycobacterium tuberculosis* of the bovine type. In the doses employed in these experiments it regularly caused the death of control guinea pigs within about a month after subcutaneous inoculation. In all experiments the test material, containing this test organism with organic matter in the form of spleen tissue or skim milk, was mixed with the disinfectant, and at the conclusion of the period of exposure portions of each test mixture were inoculated subcutaneously into guinea pigs weighing from 400 to 500 gm. Since orthophenylphenol is practically insoluble in water it was dissolved by the aid of coconut-oil soap or by the use of sodium hydroxide or calcium hydroxide. The amounts of sodium hydroxide or calcium hydroxide were such as to yield the neutral sodium or calcium salt of orthophenylphenol.

<sup>1</sup> Received for publication Jan. 28, 1931; issued May, 1931.

<sup>2</sup> The bacteriological nomenclature used in this paper is that of the following publication: BERGEY, D. H. BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY; A KEY FOR THE IDENTIFICATION OF ORGANISMS OF THE CLASS SCHIZOMYCETES . . . Ed. 3, 589 p., Baltimore. 1930.

In experiment 1 (Table 1) the test material was a suspension prepared by grinding in normal saline solution the spleen of a guinea pig infected by the previously mentioned strain of *Mycobacterium tuberculosis*. Autopsy showed extensive generalized tuberculosis and microscopic examination of the spleen suspension showed the presence of many tubercle bacilli. After the suspension was prepared it was allowed to stand long enough for large particles to settle out. At the time it was used it had a density approximately equal to that of a vigorous bouillon culture of *Eberthella typhi*. Portions of the spleen suspension were mixed with equal volumes of disinfectant solutions of different concentrations and after two minutes' exposure 1 c. c. portions of the various test mixtures were inoculated subcutaneously into guinea pigs. Control animals received  $\frac{1}{2}$  c. c. of spleen suspension.

In all other experiments (Table 1) the test material was a suspension of a culture of *Mycobacterium tuberculosis*, grown on glycerinated beef infusion broth, in  $\frac{M}{15}$   $\text{Na}_2\text{HPO}_4$ . At the time it was used this suspension had a density approximately equal to that of a vigorous bouillon culture of *Eberthella typhi*. The test mixtures contained 50 per cent of sterile skim milk, 25 per cent of culture suspension, and 25 per cent of disinfectant solution of different concentrations. At the end of the periods of exposure shown in the table, 2-c. c. portions of each test mixture were inoculated subcutaneously into guinea pigs. Control animals received  $\frac{1}{2}$  c. c. of culture suspension.

TABLE 1.—*Germicidal efficiency of orthophenylphenol against Mycobacterium tuberculosis as determined by subcutaneous inoculation of guinea pigs*<sup>a</sup>

EXPERIMENT 1<sup>b</sup>

Guinea pig No.	Concentrations in test mixtures			Time of exposure	Results of guinea-pig inoculations	
	Ortho-phenyl-phenol	Coconut-oil soap	Organic matter and test organism		Termination of test <sup>c</sup>	Lesions of tuberculosis found
1	1-200	1-100	50 per cent spleen suspension containing test organism.	2	Killed after 72 days	None.
2	1-200	1-100		2	do	Do.
3	1-400	1-200		2	do	Generalized.
4	1-400	1-200		2	do	None.
5	1-800	1-400		2	Died of injury after 18 days.	Slight.
6	1-800	1-400		2	Died after 65 days	Generalized.
7	1-200	1-20		2	Died after 60 days	Do.
8	1-200	1-20		2	Died after 35 days	Do.

EXPERIMENT 2<sup>d</sup>

9	1-200	1-100	50 per cent skim milk; 25 per cent culture suspension.	2	Killed after 142 days	None.
10	1-200	1-100		2	do	Do.
11	1-400	1-200		2	Died after 54 days	Generalized
12	1-400	1-200		2	Died after 106 days	Do.

<sup>a</sup> Experiments conducted at room temperature; weight of guinea pigs 400 to 500 gms.

<sup>b</sup> 1 c. c. of test mixture inoculated into test guinea pigs;  $\frac{1}{2}$  c. c. into controls.

<sup>c</sup> Life of controls was approximately 30 days

<sup>d</sup> 2 c. c. of test mixture inoculated into test guinea pigs,  $\frac{1}{2}$  c. c. into controls.

TABLE 1.—Germicidal efficiency of orthophenylphenol against *Mycobacterium tuberculosis* as determined by subcutaneous inoculation of guinea pigs—Contd.EXPERIMENT 3<sup>a</sup>

Guinea pig No.	Concentrations in test mixtures			Time of exposure	Results of guinea-pig inoculations	
	Ortho-phenyl-phenol	Coconut-oil soap	Organic matter and test organism		Termination of test	Lesions of tuberculosis found
13	1-200	1-100	50 per cent skim milk, 25 per cent culture suspension.	2	Killed after 182 days....	None.
14	1-200	1-100		2	Died of intercurrent disease after 102 days.	Do.
15	1-400	1-200		2	Died after 57 days.....	Generalized.
16	1-400	1-200		2	Died after 56 days.....	Do.

EXPERIMENT 4<sup>a</sup>

17	1-200	1-100	50 per cent skim milk; 25 per cent culture suspension	2	Died of intercurrent disease after 89 days.	None.
18	1-200	1-100		2	Killed after 124 days....	Do.

EXPERIMENT 5<sup>a</sup>

19	1-600	1-300	50 per cent skim milk; 25 per cent culture suspension.	30	Killed after 61 days....	Generalized
20	1-600	1-300		30	Do.	Do.
21	1-600	1-300		60	Died after 35 days....	Do.
22	1-600	1-300		60	Died after 56 days....	Do.

EXPERIMENT 6<sup>a</sup>

23	1-400	1-200	50 per cent skim milk; 25 per cent culture suspension.	15	Died of intercurrent disease after 46 days.	None.
24	1-400	1-200		15	Killed after 124 days....	Do.
25	1-400	1-200		30	Do.	Do.
26	1-400	1-200		30	Do.	Do.
27	1-400	1-200		60	Do.	Do.
28	1-400	1-200		60	Died of intercurrent disease after 70 days	Do.
29	1-600	1-300		60	Killed after 124 days....	Do.
30	1-600	1-300		60	Do.	Do.

EXPERIMENT 7<sup>a</sup>

31	1-400	(*)	50 per cent skim milk; 25 per cent culture suspension.	30	Killed after 94 days....	None.
32	1-400	(*)		30	Died of intercurrent disease after 13 days.	Do.
33	1-400	(†)		30	Died of intercurrent disease after 52 days.	Do.
34	1-400	(†)		30	Killed after 94 days....	Do.

<sup>a</sup> 2 c. c. of test mixture inoculated into test guinea pigs; ½ c. c. into controls.

\* NaOH substituted in quantities sufficient to yield neutral sodium salt.

† Ca (OH)<sub>2</sub> substituted in quantities sufficient to yield neutral calcium salt.

## DISCUSSION

The results given in Table 1 show that after two minutes' exposure orthophenylphenol in a final concentration of 1-200 with a soap concentration of 1-100 was uniformly effective in mixtures containing tubercle bacilli and in the presence of a high percentage of organic matter. The bacilli, as previously noted, were present in large numbers. With the same technic, lower concentrations of orthophenylphenol or the same concentration with a much higher concentration



of soap were not effective. If an allowance of 50 per cent is made for dilution of the disinfectant under practical disinfecting conditions, the foregoing results, together with those reported in a previous paper,<sup>3</sup> suggest that a solution containing 1 per cent of orthophenylphenol and 2 per cent of coconut-oil soap should be effective where rapid action is desired as, for example, in disinfecting the hands.

Results given in the table suggest that for general disinfecting purposes against *Mycobacterium tuberculosis*, where longer exposure is possible, orthophenylphenol in a final concentration of 1-200, dissolved by the aid of coconut-oil soap, sodium hydroxide, or calcium hydroxide, should be effective. Other work, as yet unpublished, indicates that an excess of sodium hydroxide or calcium hydroxide tends to diminish germicidal activity. It is advisable, therefore, to use only as much of these alkalies as may be necessary to dissolve the orthophenylphenol.

#### SUMMARY

Tests were made with solutions of orthophenylphenol prepared by the aid of coconut-oil soap, sodium hydroxide, or calcium hydroxide, against *Mycobacterium tuberculosis*. After two minutes' exposure orthophenylphenol in a concentration of 1-200 was effective with a soap concentration of 1-100 but was not effective with a soap concentration of 1-20, in the presence of a considerable amount of organic matter. Under similar conditions a concentration of 1-400 with a soap concentration of 1-200 was not effective. Solutions containing orthophenylphenol in a concentration of 1-400 with coconut-oil soap in a concentration of 1-200 or with just enough sodium hydroxide or calcium hydroxide to yield the neutral sodium or calcium salt of orthophenylphenol were uniformly effective after 30 minutes' exposure. Solutions containing orthophenylphenol in a concentration of 1-600 and coconut-oil soap in a concentration of 1-300 were sometimes effective (experiment 7) and sometimes ineffective (experiment 5) after 60 minutes' exposure.

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<sup>3</sup> SCHAFER, J. M., and TILLEY, F. W. GERMICIDAL EFFICIENCY OF SOAP AND OF MIXTURES OF SOAPS WITH SODIUM HYDROXIDE OR WITH PHENOLS. Jour. Agr. Research 11: 737-747. 1930.

# THE EFFECT OF SALT ON THE MICROBIAL HEATING OF ALFALFA HAY<sup>1 2</sup>

By L. S. STUART, *Associate Bacteriologist*, and LAWRENCE H. JAMES, *Senior Bacteriologist*, Bureau of Chemistry and Soils, United States Department of Agriculture

## INTRODUCTION

During a study of the heating of moistened hay in the flooded regions of Vermont in 1927 (6),<sup>3</sup> reports were received from many farmers who had had marked success in preventing the molding and spontaneous heating of stored hay with a high moisture content by the application of large quantities of salt. No information was available at that time, however, as to the actual rôle of the salt in preventing heating, or as to the quantities which could be used safely without causing injury to livestock. Laboratory studies were undertaken, therefore, to determine the reason for the effect of salt on the microbial heating of organic material.

James, Rettger, and Thom (7), who studied heat production in moist organic materials by microorganisms, found that the preliminary heat produced during spontaneous heating was in a large part due to microbial activity. That sodium chloride inhibits the growth of certain microorganisms is well known (4). In fact, Duthoit (1, 2, 3) and others have demonstrated bactericidal action in salt solutions. Shutt (9) observed that hay to which salt had been added during the storing process in every case appeared brighter than hay stored unsalted. However, no appreciable chemical differences were noted between salted and unsalted hays. Feeding experiments showed that 20 pounds of salt per ton could be added without bad effects on the animals, but when 30 pounds per ton were added marked physiological effects were noted.

## EXPERIMENTAL WORK

James (5) devised an apparatus for the study of heat production by microorganisms in moist organic material. This apparatus was adopted for the studies reported here with the exception that air was used as the source of oxygen. The heating flasks were not subjected to forced aeration, but were allowed to draw air freely through the aerating tubes.

To prove that heating which occurred without forced aeration was actually dependent upon oxidation, three flasks were packed with equal quantities of chopped alfalfa hay and rubber policemen fitted to prevent the passage of air through the aerating tubes. To the hay in one (Dewar No. 1) water was added to bring the final moisture content to 40 per cent. To the hay in the other two flasks (Dewar Nos. 2 and 3) salt solutions were added to bring the final moisture content to 40 per cent, and the salt concentrations to 1 and 2 per cent, respectively. The quantity of salt used was determined

<sup>1</sup> Received for publication Dec. 22, 1930; issued May, 1931.

<sup>2</sup> This paper is a joint contribution from the divisions of Chemical Engineering and Food Research, Chemical and Technological Research, Bureau of Chemistry and Soils.

<sup>3</sup> Reference is made by number (*italic*) to Literature cited, p. 664.

from the total weight of the hay and the water to be added. No significant heating occurred during a period of eight days. (Table 1.)

TABLE 1.—Heat produced during eight days by moistened<sup>a</sup> chopped alfalfa hay, packed in insulated Dewar flasks, with and without salt, and not aerated

Days packed	Hour observed	Temperature in—			
		Room	Flask No. 1, no salt	Flask No. 2, 1 per cent salt	Flask No. 3, 2 per cent salt
		°C.	°C.	°C.	°C.
0	12 m.	29.0	28.0	28.0	28.0
	4 p. m.	29.5	27.5	27.5	27.5
	9 a. m.	29.5	29.0	28.0	29.0
1	12 m.	28.0	30.0	29.0	29.0
	4 p. m.	30.0	30.0	29.5	30.0
	9 a. m.	30.0	32.0	31.0	31.0
2	12 m.	31.0	31.5	31.0	31.5
	4 p. m.	30.5	34.5	31.0	31.0
	9 a. m.	29.5	31.0	30.5	31.0
3	12 m.	30.0	31.0	29.0	31.0
	4 p. m.	31.0	31.0	29.5	31.0
8	9 a. m.	31.0	31.0	31.0	31.0

<sup>a</sup> 40 per cent moisture.

These results confirm the observations of other investigators, namely, that heating does not take place in the absence of air or oxygen. The macroscopic appearance of the moistened hay in the Dewar flasks was unchanged except for a slight mold growth on the surface, which penetrated the material about 3 inches.

TABLE 2.—Heat produced during six days by moistened<sup>a</sup> chopped alfalfa hay packed in insulated Dewar flasks, with and without salt, and aerated

Days packed	Hour observed	Temperature in—			
		Room	Flask No. 1, no salt	Flask No. 2, 1 per cent salt	Flask No. 3, 2 per cent salt
		°C.	°C.	°C.	°C.
0	10 a. m.	24.5	24.5	24.5	24.5
	4 p. m.	24.5	24.5	24.0	24.0
	9 a. m.	25.0	26.0	24.5	24.5
1	12 m.	25.0	26.0	24.5	24.5
	4 p. m.	27.0	26.0	24.5	24.5
	9 a. m.	25.5	28.5	21.5	24.0
2	12 m.	25.5	28.5	23.5	24.0
	4 p. m.	25.0	30.5	23.5	23.0
	9 a. m.	25.0	41.0	23.5	23.5
3	12 m.	27.0	43.0	25.0	23.5
	4 p. m.	24.0	45.5	25.5	23.0
	9 a. m.	27.0	47.0	26.5	23.0
4	12 m.	26.0	46.5	40.0	23.5
	4 p. m.	26.0	47.5	41.5	25.0
	9 a. m.	25.0	44.5	44.5	31.0
5	12 m.	26.0	44.0	44.0	33.0
	4.30 p. m.	26.0	44.0	44.0	37.0
	9 a. m.	27.0	44.0	44.5	43.0
6	12 m.	26.0	43.5	47.0	45.0
	4.30 p. m.	25.0	44.5	47.0	44.5

<sup>a</sup> 40 per cent moisture.

To confirm the supposition that the flasks would take in enough air for heating without forced aeration, and under such conditions to observe the effect of adding 1 and 2 per cent salt to moist hay, three

flasks were packed with equal quantities of alfalfa hay which had been treated in the same manner as in the preceding experiment. Heating was observed in all flasks, although where 1 per cent salt had been added, heating did not start so soon as in the absence of salt, and where 2 per cent salt had been added heating was further delayed. At the end of six days the maximum temperatures in the three flasks were about the same. (Table 2.) At the conclusion of the heating tests the material in all three flasks contained large quantities of mold growth.

As James, Rettger, and Thom (7) have shown that the quantity of moisture markedly affects the rate of heat development, a series of experiments was conducted in which flasks were packed with equal quantities of alfalfa hay containing varying percentages of added moisture, without salt, and with 1 and 2 per cent salt, respectively. (Table 3.) With 15 per cent moisture, which was the lowest moisture used, 2 per cent added salt was not sufficient to prevent heating, although it did not start until the ninth day, whereas with no salt heating started on the fourth day. With 20 per cent added moisture and no salt, heating occurred within four days, whereas heating did not appear until the sixth day in the presence of 1 per cent salt, and not until the ninth day where 2 per cent salt had been added.

TABLE 3.—*Effect of varying moisture content on the rate of heat development in moistened alfalfa hay, with and without the addition of salt*<sup>a</sup>

Added moisture (per cent)	Heat development in the presence of—					
	No salt		1 per cent salt		2 per cent salt	
	Time	Maximum temperature	Time	Maximum temperature	Time	Maximum temperature
	Days	° C.	Days	° C.	Days	° C.
15.....	4	50.0	9	48.5	9	45.0
20.....	4	43.5	6	44.0	9	43.0
25.....	2	45.0	5	44.0	8	42.5
30.....	2	48.0	4	48.0	6	44.5
35.....	2	41.0	3	41.0	4	41.0
40.....	2	47.5	3	44.0	5	44.5

<sup>a</sup> Data for this table were compiled in a manner similar to that reported in Table 2.

With 25 per cent or more added moisture and no salt the material invariably started to heat within two days. When 1 per cent salt was added heating started on the fifth day with 25 per cent moisture, on the fourth day with 30 per cent moisture, and on the third day with 35 and 40 per cent moisture. With 2 per cent added salt heating began on the eighth day with 25 per cent moisture, on the sixth day with 30 per cent, on the fourth day with 35 per cent, and on the beginning of the fifth day with 40 per cent.

The maximum temperatures seemed not to be materially affected by the addition of salt. Heat development was more rapid with 30 per cent moisture than with lower percentages of moisture and no addition of salt. This finding substantiates the results obtained in previous investigations.

The results of these experiments indicate that the action of 1 and 2 per cent salt is affected by the quantity of moisture present, namely, with equal quantities of salt, the greater the percentage of moisture the more quickly heating takes place.

The possibility that dry salt may have a greater efficacy in delaying or preventing heat production than the addition of salt in solution was investigated. Alfalfa hay was moistened to a moisture content of 40 per cent. Three flasks were packed with equal quantities. One per cent of salt was added to flask No. 2 and 2 per cent of salt to flask No. 3. The dry salt was sprinkled evenly over the material. The results of this test are given in Table 4.

TABLE 4.—Heat produced during five days by moistened<sup>a</sup> chopped alfalfa hay, packed in insulated Dewar flasks, with 1 and 2 per cent salt added dry

Days packed	Hour observed	Temperature in—			
		Room	Flask No. 1, no salt	Flask No. 2, 1 per cent salt	Flask No. 3, 2 per cent salt
		° C.	° C.	° C.	° C.
0.	12.00 m.	31.5	31.5	31.5	31.5
	4.30 p. m.	32.0	32.0	31.5	31.5
1.	9.00 a. m.	31.5	31.5	31.5	31.5
	12.00 m.	32.0	31.5	31.5	31.5
2.	4.30 p. m.	32.0	31.5	31.5	31.0
	9.00 a. m.	29.0	32.5	32.0	32.0
3.	12.00 m.	29.0	32.5	32.0	32.0
	4.30 p. m.	31.0	33.0	32.0	31.5
4.	9.00 a. m.	30.5	45.0	36.0	34.0
	12.00 m.	31.0	45.0	37.0	34.0
5.	4.30 p. m.	31.0	45.0	38.0	31.0
	9.00 a. m.	29.5	45.0	47.5	39.0
6.	12.00 m.	30.0	41.5	47.0	40.0
	4.30 p. m.	30.5	45.0	46.0	41.0
7.	9.00 a. m.	27.0	46.0	46.5	43.0

<sup>a</sup> 40 per cent moisture.

No difference could be observed between the action of salt when added in a dry form and in solution. Mische (3, p. 116-122) attributed the greater heating propensity of dung piles that were treated with kainite and mixtures of superphosphate and gypsum in part to the desiccation which resulted from the absorption of water by the salts. The addition of dry salt to large piles of moist or partly moist organic material might have considerable effect upon heating due to the desiccation and possible distribution of moisture throughout the mass. The laboratory heating tests reported in this paper were not large enough to demonstrate such possible physical activities.

Microbial analyses were made of heating alfalfa hay containing 1 and 2 per cent salt, respectively. Bacterial counts were made on the original and test materials on plain agar, plain agar plus 5 per cent salt, and wort agar. (Tables 5 and 6.) In the heating flasks to which no salt had been added large increases of aerobic spore-forming bacteria and molds were noted on plain agar. In many of the flasks to which 1 per cent salt had been added an increase in the numbers of the aerobic spore-forming bacteria occurred, but mold growth seemed to predominate.

TABLE 5.—*Bacterial plate counts from chopped and moistened alfalfa hay, salted and unsalted, that had developed heat*

Salt added to hay	Maximum temperature of heating hay	Incubation period	Total counts on plain agar at 37.5° C.	Total counts on 5 per cent salt agar at 37.5° C.	Total counts on wort agar at 30° C.
Per cent	° C.	Days			
0	27.5	0	<sup>a</sup> 310,000	<sup>b</sup> 1, 140,000	<sup>c</sup> 560
0	45.0	3	<sup>a</sup> 520,000	<sup>b</sup> 580,000	(d)
0	45.0	4	<sup>a</sup> 100,000	<sup>b</sup> 668,000	(d)
0	46.0	6	<sup>a</sup> 1,000,000	<sup>b</sup> 8,000,000	(d)
1	36.0	3	<sup>a</sup> 540,000	<sup>b</sup> 540,000	(d)
1	47.5	4	<sup>a</sup> 6,480,000	<sup>b</sup> 7,280,000	(d)
1	46.5	6	(c)	<sup>b</sup> 19,680,000	(d)
2	34.0	3	<sup>a</sup> 32,400	<sup>b</sup> 242,000	(d)
2	39.0	4	<sup>a</sup> 120,000	<sup>b</sup> 146,000	(d)
2	43.0	6	<sup>a</sup> 140,000	<sup>b</sup> 30,560,000	<sup>b</sup> 7,200,000

<sup>a</sup> Subtilislike colonies.<sup>b</sup> Pin-point colonies only.<sup>c</sup> Species of *Aspergilli* and *Penicillia*.<sup>d</sup> Plates covered with species of *Aspergilli* and *Penicillia*.<sup>e</sup> Plates covered 1:1,000,000 dilution.

In the flasks to which 2 per cent salt had been added the results were somewhat different. In every case the plain agar counts from this material were less than those on the original unpacked hay. This was true even after the flasks had been held for as long as 9 to 10 days. Salt agar plates from the original material gave high counts of pin-point colonies, which proved on examination to be largely gram-positive cocci and bacilli. Increases in the counts on salt agar did not occur until after heating had been continued over a prolonged period. It would seem, therefore, that such increases are not associated with the actual rise in temperature, but rather are a result of the incubation effect produced by the growth of other organisms. However, it is possible that the action of these microorganisms assisted in maintaining and prolonging high temperatures. Wort agar plates showed marked increases in the number of mold colonies as heating progressed.

The delay in heating in the flask that contained 1 per cent salt as compared with the flask to which no salt was added can not definitely be accounted for, although the results of the experiments would seem to show that it was in part owing to the inhibitive effect of salt on certain bacteria and in part to an increase in the "lag" period of the salt-tolerant microorganisms present.

The increased delay in the flasks containing 2 per cent salt could then be attributed to an increased efficiency of the same action. In fact, results would seem to indicate that 2 per cent salt entirely eliminates the factor of heating from bacterial action, leaving only the heating from the mold growth.

The data in Table 6 show a marked difference in the microbial counts during heating, suggesting variations in the types of organisms present. In order to obtain more definite information relative to the effect of salt in modifying the microbial flora, four flasks were packed with moistened chopped alfalfa hay, and salt was added to two flasks, 1 and 2 per cent, respectively. Examinations were made in the first before heating began, and in the other three when a temperature of 41° C. had been reached. The results given in Table 6 show that anaerobes are present in fair quantities throughout; that lactose ferments and gelatin liquefiers and other organisms which grew at 20° are inhibited by salt; that plain agar and 5 per cent salt agar counts are similar to those given in Table 5; and that yeasts and molds markedly increase in all cases.

TABLE 6.—*Microbial analyses of heating chopped moistened alfalfa hay with and without the addition of salt*<sup>a</sup>

Salt added to hay	Incubation period	Maxi-mum temperature of heating hay	Total counts when cultured in—							
			Lactose broth at 37.5° C.	Anaerobic beef broth at 37.5° C.	(gelatin at 20° C.	Plain agar at 37.5° C.	Plain agar at 37.5° C. (Spore count <sup>b</sup> )	5 per cent salt agar at 37.5° C.	Dextrose agar at 37.5° C.	Wort agar at 30° C.
Per cent	Days	° C.								
0	0	26.5	Gas in 0.1 dilution.....	Putrefactive odor in 0.1 dilution.	202,000.....	ε 810,000	ε 152,000	d 16,400	ε 302,000	ε 38,000
0	3½	41.0	do.....	do.....	Liquefiers, 1,420. 30,000.....	ε 17,000,000	ε 23,600,000	d 480,000	ε 20,400,000	ε 4,400,000
1	4	41.0	do.....	do.....	Liquefiers, 10,000. No growth in 48 hours.	ε 2,400,000	d 280,000	d 180,000	ε 320,000	ε 3,800,000
2	6	41.0	No gas in 0.1 dilution..	do.....	do.....	d 64,000	d 44,000	Mold growth only.	28,000 (Few molds)	ε More than 10,000,000.

<sup>a</sup> Counts made on water washings of 10-gram samples, recorded as counts per gram.<sup>b</sup> Spore counts made by heating sample to 80° C. for 20 minutes, immersing quickly in cold water and plating.<sup>c</sup> Subtilis and mycoideslike colonies.<sup>d</sup> Pin-point colonies only.<sup>e</sup> Species of *Aspergillus*, *Penicillium*, and *Oidium*.

All flasks that had heated contained large quantities of mold growth. An experiment was now conducted to determine, if possible, the quantity of salt necessary to prevent molding and subsequent heating. Three flasks were packed, containing no salt, 4 per cent salt, and 5 per cent salt, respectively. The results of this experiment showed that 5 per cent salt per total weight of hay (containing 30 per cent moisture) was not sufficient to prevent molding and heating. (Table 7.) The flasks showed a heavy mold growth.

TABLE 7.—*Heat produced during 13 days by moistened<sup>a</sup> chopped alfalfa hay, packed in insulated Dewar flasks, with 4 and 5 per cent salt*

Days packed	Hour observed	Temperature in—			
		Room	Flask No. 1, no salt	Flask No 2, 4 per cent salt	Flask No. 3, 5 per cent salt
		°C.	°C.	°C.	°C.
1.....	10 00 a. m.....	27.0	27.0	27.0	27.0
2.....	{ 9.00 a. m.....	27.5	27.5	27.0	27.0
	{ 4.00 p. m.....	27.0	27.5	26.5	27.0
3.....	{ 9.00 a. m.....	25.0	26.5	26.0	26.0
	{ 12.00 m.....	26.0	26.5	26.0	26.0
4.....	{ 9.00 a. m.....	24.0	27.0	26.0	26.0
	{ 9.00 a. m.....	25.0	15.5	27.0	27.0
7.....	{ 12.00 m.....	26.0	45.0	27.0	27.0
	{ 4.00 p. m.....	27.0	44.5	27.0	27.0
8.....	{ 12.00 m.....	32.0	46.0	30.0	30.0
	{ 4.00 p. m.....	29.5	46.0	29.5	29.5
	{ 9.00 a. m.....	26.5	46.0	29.5	29.5
9.....	{ 12.00 m.....	26.0	46.0	29.5	29.5
	{ 4.00 p. m.....	28.5	45.5	29.5	29.5
	{ 9.00 a. m.....	30.5	46.0	30.0	30.5
10.....	{ 12.00 m.....	29.0	47.5	32.5	30.5
	{ 4.30 p. m.....	29.0	47.5	32.5	30.5
	{ 9.00 a. m.....	29.5	49.0	39.0	34.0
11.....	{ 12.00 m.....	29.0	48.5	41.0	34.5
12.....	{ 9.00 a. m.....	29.0	48.5	43.5	42.5
13.....	{ 9.00 a. m.....	28.5	47.0	46.0	44.5

<sup>a</sup> 30 per cent moisture.

Since even 5 per cent salt would be far in excess of the 30 pounds per ton found to have a marked physiological effect upon cattle (9), it would seem that the quantity of salt necessary to prevent molding would be far in excess of the quantity that could safely be added to hays under actual practical conditions. However, addition of salt to slightly damp and spotted hays might delay heating until the moisture content had lowered, through evaporation or equalization, below the critical point for mold growth. This could be demonstrated effectively only in large-scale experiments.

#### SUMMARY

Moistened alfalfa hay packed in a Dewar flask into which an aerating tube had been inserted and left open heated to 47.5° C. When the aerating tubes were closed, no heating was observed.

Moistened alfalfa hay to which 1 per cent salt had been added did not start to heat so soon as hay to which no salt had been added, and when 2 per cent salt was added heating did not start so soon as when 1 per cent salt had been added.

Flasks filled with alfalfa hay with different moisture contents, with no salt and with 1 and 2 per cent salt, showed that with 30 per cent moisture and no salt, heat development was more rapid than with



lower percentages of moisture, and that the delay in heating when salt was added varied inversely with the quantity of moisture present.

No difference was noted between the action of dry salt and salt in solution.

Bacteriological examination (1) of unsalted hay at various stages of heating showed marked increases in mold and aerobic spore-forming bacteria as the heating progressed, (2) of hay to which 1 per cent salt had been added showed marked increases in mold growth as heating progressed, and (3) of hay to which 2 per cent salt had been added showed marked decreases in bacterial counts and increases in mold growth as heating progressed.

Alfalfa hay containing 5 per cent salt and 30 per cent moisture molded and heated within 12 days.

Salt added to moistened alfalfa hay will inhibit bacterial growth and will delay but not prevent mold development. This delay in microbial development may be long enough to permit the curing of the hay.

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# A PRELIMINARY STUDY OF THE DETERMINATION OF THE APPARENT DIGESTIBILITY OF PROTEIN BY MODIFIED PROCEDURES<sup>1</sup>

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## INTRODUCTION

Modifications of the usual methods employed in the determination of the apparent digestibility of food are of practical importance in so far as they yield more accurate results or simplify technical details relative to such determinations. A recent modification has been suggested by Bergeim (1),<sup>2</sup> who recommends the addition to the food of iron oxide or other suitable substance which is excreted with practical completeness in the feces. The digestibility coefficients are then calculated from a determination of the ratio of food substance to iron in the diet and as found in the feces. This method offers simplicity, since a measure of the amount of food consumed and feces excreted during an experimental period is not necessary. Its precision as a method for determining the composition of feces from which digestibility data are obtained appears to be limited to the accuracy of the iron determinations and the maintenance of a constant ratio between iron and food constituents in the diet.

In a previous study of the digestibility of protein, in which this method was employed, one of the authors (4) obtained results comparable to those obtained by the usual method, in which the total amount of food eaten and that excreted was determined and the digestibility calculated by difference. Heller, Breedlove, and Likely (7) believed that greater accuracy could be attained with Bergeim's method if the normal iron content of the diet were used as a reference substance, since practically all of the iron appears in the feces and any errors produced through an uneven distribution of the iron oxide in the food are eliminated. Other experiments (5) have demonstrated the practicability of substituting silica for iron oxide under conditions in which the iron can not be recovered quantitatively from the feces.

All of the foregoing experiments have been conducted with rats as experimental animals and under conditions in which a measure of the food eaten and the daily collection of feces have not presented great difficulty. Bergeim (1, p. 32), in commenting on his proposed method, states: "The extent to which this procedure may replace the usual method can only be demonstrated by experiments carried out under a variety of conditions." The results obtained in the following experiment indicate the extent to which modified procedures may be employed in digestibility studies with large animals where all the difficulties of the older method of experimentation pre-

<sup>1</sup> Received for publication Jan. 19, 1931; issued May, 1931.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 669.

vail. The limited value of digestibility coefficients obtained in this way has been fully discussed by Mitchell (8).

### EXPERIMENTAL DATA

The usual precautions necessary in conducting a digestibility study were observed throughout the experiment. The experimental animals were four Jersey heifers approximately  $1\frac{1}{2}$  years old and weighing about 500 pounds. The rations were similar to those being used in nutrition studies with cottonseed meal. Their composition is presented in Table 1 in such a manner as to show the daily food allowance.

TABLE 1.—*Composition of rations used in nutrition studies with cottonseed meal*

[NaCl was fed ad libitum]

Constituent	Quantity in ration No. <sup>a</sup> -			
	21	22	25	26
Cottonseed meal.....pounds.....	5.5	5.5	---	---
Cottonseed meal (autoclaved).....do.....	---	---	5.5	5.5
Prairie hay (chopped).....do.....	8.0	8.0	8.0	8.0
CaCO <sub>3</sub> .....grams.....	---	15.0	---	15.0
Fe <sub>2</sub> O <sub>3</sub> <sup>b</sup> .....do.....	13.0	13.0	13.0	13.0

<sup>a</sup> This number also refers to the laboratory number of the animal. In reality only two rations were made up since the CaCO<sub>3</sub> was added at the time of feeding.

<sup>b</sup> Approximate values are given for Fe<sub>2</sub>O<sub>3</sub>.

Choice cottonseed meal containing 43 per cent crude protein was used in these experiments. The autoclaved meal was prepared in the laboratory by cooking the meal under 25 pounds of steam pressure for 30 minutes. This process, which is effective in destroying much of the gossypol that may be present in the commercial meal, has been discussed elsewhere (4).

The feeding trial lasted 10 days, during which time four samples of each ration were taken for proximate analysis and iron determinations. The results of the analyses are presented in Table 2. Samples of fresh feces were collected in the morning and evening during five days of the experiment. Composite samples of the last three daily collections were used for the calculation of the coefficients of digestibility. The results are presented in Table 3, in which periods 1, 2, and 3 represent the last three days of the experiment. Iron determinations were made by following a colorimetric method outlined by Bergeim (2), special care being exercised in dissolving the iron and maintaining a constant acidity in all of the solutions. The results, which are presented in Table 3, are the average values obtained from four or more analyses. The difficulties encountered in using this method for the determination of fecal iron were of the same nature as have been previously reported (4, 7), and are accounted for as being due to interfering substances which were present in relatively greater amounts in the feces than in the feed.

To secure what appears to be more reliable data, the silica content of the feed and feces was determined and the digestibility coefficients calculated from the ratios so obtained. Greenwald and Gross (6) have used a similar method for studying the utilization of calcium

and phosphorus. It has been reported (3) that Wiedt fed sheep wheat straw and used the silica naturally contained therein as an index of the absorption of other substances.

TABLE 2.—Iron determinations and proximate analyses of rations expressed in percentage

Ration Nos.	Iron found in ration sample No —					Proximate analysis of rations				Silica
	1	2	3	4	Average	Protein	Nitrogen-free extract	Fat	Fiber	Average
21-22.....	0.137	0.145	0.148	0.145	0.144	18.40	42.50	4.10	22.10	3.21
25-26.....	.115	.118	.116	.114	.116	18.23	41.94	3.54	22.83	3.23

TABLE 3.—Protein, iron, and silica: Feces and results obtained by two different methods of calculating the apparent digestibility of the protein contained in similar rations

RATION 21					
Period No.	Protein in feces	Iron in feces	Silica in feces	Digestibility coefficient by—	
				Iron method	Silica method
1.....	<i>Per cent</i> 11.84	<i>Per cent</i> 0.320	<i>Per cent</i> 7.24	71.1	71.5
2.....	11.64	.273	6.81	66.7	70.2
3.....	11.45	.318	6.67	71.8	70.1
RATION 22					
1.....	12.56	0.364	8.17	73.0	73.2
2.....	11.78	.336	7.73	72.3	73.5
3.....	11.78	.260	6.88	64.3	70.1
RATION 25					
1.....	15.15	0.316	7.95	69.5	66.3
2.....	16.44	.331	9.50	68.4	69.9
3.....	13.23	.295	7.20	71.5	67.5
RATION 26					
1.....	14.40	0.350	7.48	73.8	66.6
2.....	13.37	.250	6.41	65.6	63.1
3.....	13.93	.288	8.18	68.6	69.8

## DISCUSSION

An even distribution of iron oxide through the ration is of primary importance, and the figures presented in Table 2 are taken as attesting to the completeness with which such a distribution may be made. There were no uneaten food residues, and since an examination of the containers after feeding did not show a separation of the added iron, it was assumed that the animals were eating a ration in which

the ratio of iron to other food constituents was definite and did not vary from day to day. Furthermore, analyses of feces obtained at intervals during the day showed only slight differences in composition which were in accord with the small daily variations to be expected in experiments of this kind. It appears highly probable, therefore, that variations in the digestibility coefficients which were calculated from the iron ratios and which are outside the natural limits of experimental error are caused by inaccuracies in the iron determinations. This possibility is suggested, although Bergeim (1) has stated that if 10 per cent of a given food substance is not digested a 10 per cent error in the analyses will produce only a 1 per cent change in the digestibility value. The amount of undigested substance is oftentimes much greater than this, and as a consequence small errors in the iron determinations produce conspicuous variations in the digestibility coefficients.

The lack of reliable methods for the rapid determination of iron under a variety of conditions, some of which predominate in digestibility studies, has been brought out by Heller (7) and discussed by many workers in papers dealing with iron metabolism. As a result, elaborate methods have been suggested which are highly accurate but are time consuming and for the most part do not qualify where simple technic is desired. Perhaps no one method will serve under all conditions, which, if true, precludes the possibility of outlining in detail a standard procedure for conducting digestibility studies in this manner. An investigation of other substances to be used in place of iron oxide under a variety of conditions would seem worth while.

The determination of the silica content of the feed and feces presented no difficulties, and the digestibility coefficients so calculated were in close agreement and of the same order as those calculated from the iron ratios. Because of the ease with which these determinations were made, this method gave promise of being preferable to the iron method. The coefficients so obtained, and presented in Table 4, agree fairly well with those which have been reported for the proteins in the rations used. Watkins (9) has recently reported the crude protein of a ration made up of cottonseed meal and straw with a nutritive ratio of 1:2.8 to be 75.7 per cent digestible. The nutritive ratio in the rations used by the writers was 1:2.4.

TABLE 4.—Average digestibility coefficients of various rations calculated from the silica content of the feed and feces

Ration No.	Digestibility coefficient of—			
	Protein	Fat	Nitrogen-free extract	Fiber
21.....	70.6	66.9	53.2	50.2
22.....	72.2	71.9	57.9	52.3
25.....	67.9	69.4	62.0	59.4
26.....	66.5	66.3	57.7	51.9

In an experiment with rats (4) that were receiving a diet containing 18 per cent protein derived solely from cottonseed meal, the apparent digestibility of the protein was found to be 71.5 per cent. In the experiments with dairy heifers reported here, the decrease in the digestibility of the protein produced by autoclaving the meal was not great, although in experiments with rats the digestibility was decreased nearly 10 per cent by this process. The presence of  $\text{CaCO}_3$  in the rations had no appreciable effect.

#### SUMMARY

A digestibility trial with large animals has been successfully carried out by modified procedures. The apparent digestibility of the protein was calculated from a determination of the ratio of the amount of protein to both iron and silica in the feed and in the feces. An average value of 71.9, which agrees closely with that reported in earlier work for the digestibility of cottonseed-meal protein, was obtained.

Under the conditions of the experiment, the silica naturally contained in the feed served as a better index of the digestibility of the other substances than did the iron which had been added for that purpose.

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# CHANGES IN THE SUGAR, OIL, AND GOSSYPOL CONTENT OF THE DEVELOPING COTTON BOLL<sup>1</sup>

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## INTRODUCTION

Present knowledge of the rate of formation of gossypol in cotton seeds as related to the accumulation of oil, protein, and certain other constituents of the cotton plant has been discussed and summarized in previous publications (2, 3).<sup>3</sup> The present investigation is a continuation of these studies of gossypol, and deals with the changes in sugar, oil, and gossypol which occur at different stages in the development of the cotton boll. The method of procedure used in this investigation offered a means of testing the validity of previous findings on the development of oil in the seeds and afforded an opportunity to observe chemical changes during much shorter intervals of growth than had previously been reported.

## EXPERIMENTAL PROCEDURE

One thousand cotton blossoms of the Oklahoma Triumph 44 variety which had been open for less than 2 days were marked by means of tags bearing the approximate date at which the flowers opened. This date was used to determine the age of the bolls that were collected at the end of 21 days and every 3 days thereafter. A 21-day period was selected as the time for the first collection because less mature bolls contain very little ether-soluble material. The last collection was made on the fiftieth day, when all the bolls had reached the mature stage. A description of the material so collected is included in Table 1.

The separation of burs, lint, and seeds was made in the laboratory by hand and the dry weight determined on a small sample of each. One portion of seeds was dried at a sufficiently low temperature (50° C.) to prevent the destruction of gossypol and another portion was preserved in 80 per cent alcohol for sugar determinations. Composite samples of burs and lint were similarly preserved for sugar determinations. The amount of ether-soluble extract in the seeds, which is taken as representing oil, was determined on the dry residue from the moisture determinations. Gossypol determinations were made on 40 grams of the air-dry seeds after the manner outlined by Schwartze and Alsberg (5). Reducing sugars were determined by the Shaffer-Hartman method (6) on the alcoholic extract of the above material. The extracts, after evaporation to a small volume, were taken up with water several times and finally clarified with neutral lead acetate. Excess lead was removed with potassium oxalate (4)

<sup>1</sup> Received for publication Jan. 3, 1931; issued May, 1931.

<sup>2</sup> The authors are indebted to Dr. J. E. Webster for much valuable assistance in making the sugar determinations.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 673.



and the filtered solutions made to definite volume. Aliquots of these were used for determining the reducing and total sugars. The total sugars were determined after hydrolysis with citric acid according to the procedure described by Webster and Dalbom (7). The sugar equivalents in terms of dextrose were obtained from Munson and Walker tables (1).

### EXPERIMENTAL RESULTS

The gossypol, oil, and sugar content of cotton bolls at different stages of maturity is shown in Table 1. An examination of this table discloses the fact that there is a concurrent formation of oil and gossypol in the seeds with a rapid increase of each during 10 days of growth. The relatively more rapid increase of gossypol than of oil during this early period of growth is brought out by calculating the ratio of oil to gossypol which decreases from 159:1 at 21 days to 58:1 at 34 days and does not fall below 50:1 thereafter. The critical period of oil formation appears to be between the twenty-first and thirtieth day. The sugar changes do not lend themselves to interpretation in relation to gossypol but show a more gradual and continued decrease as the seeds mature. Nor do the figures give convincing evidence of the formation of oil at the expense of sugar, except as they show a decrease in sugars as the oil increases.

TABLE 1.—*Gossypol, oil, and sugar content of separate parts of the cotton boll at different stages of maturity*

SEED						
Age, days	Moisture	Dry weight from 100 bolls	Percentage content of various constituents on a dry-matter basis			
			Oil	Reducing sugar <sup>a</sup>	Total sugar <sup>a</sup>	Gossypol
	Per cent	Grams				
21.....	75.2	174	8.26	2.78	4.60	0.052
24.....	73.5	177	11.73	2.90	3.20	.113
27.....	70.0	225	16.66	1.97	2.30	.168
30.....	65.0	246	20.55	1.57	1.86	.266
34.....	56.8	302	20.25	.81	1.23	.347
37.....	52.0	316	20.80	.56	1.08	.360
41.....	26.6	386	<sup>b</sup> 22.30	.16	.42	.356
44.....	17.7	329	20.20	.12	.32	.399
50.....	23.2	274	20.90	.15	.32	.408
LINT						
21.....	70.25	132		11.35	10.98	
24.....	70.25	137		7.90	8.57	
27.....	65.8	141		7.60	7.80	
30.....	58.5	222		3.43	3.55	
34.....	47.5	241		2.89	3.40	
37.....	48.5	205		1.94	2.30	
41.....	17.0	208		.41	.55	
44.....	12.0	205		.23	.38	
50.....	12.0	202		.17	.28	
BURS						
21.....	80.85	164		3.67	3.75	
24.....	82.61	142		<sup>b</sup> 6.73	<sup>b</sup> 7.00	
27.....	80.66	144		3.69	4.77	
30.....	80.50	159		3.90	4.61	
34.....	78.00	184		2.37	3.05	
37.....	80.00	138		2.00	2.93	
41.....	65.00	144		.96	1.05	
44.....	49.00	154		.58	.69	
50.....	18.40	154				

<sup>a</sup> Calculations in terms of dextrose.

<sup>b</sup> Values are probably incorrect.

## SUMMARY

The sugar, oil, and gossypol content of cotton bolls at different stages of maturity was determined. Both gossypol and oil increased rapidly in the seed from the twenty-first day until the thirtieth day. The gossypol increased more rapidly than the oil, and continued to increase slowly until the boll was 50 days old and fully matured. The sugars in all parts of the boll decreased gradually during the 30-day period of growth.

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# THE EFFECT OF PASTEURIZING AND HOMOGENIZING TEMPERATURES ON CERTAIN PROPERTIES OF ICE-CREAM MIXES<sup>1</sup>

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## INTRODUCTION

Standard or uniform pasteurization temperatures have never been adopted by the ice-cream industry; consequently the temperatures used vary widely. The same may be said of the temperatures employed for the homogenization of the mix, for in the majority of cases the mixes are homogenized at the pasteurizing temperature. The lack of uniform standards prompted this study.

In the market-milk industry the use of pasteurizing temperatures between 142° and 145° F. for a period of 30 minutes is the rule. Following the example set by the market-milk industry, ice-cream manufacturers until recent years have pasteurized the mix at a temperature of about 145° and held it at this temperature for a period of 30 minutes. This temperature was considered effective for the killing of pathogenic bacteria. Hammer and Sanders (6)<sup>2</sup> found considerable destruction of bacteria at temperatures of 142° to 150°. Fabian and Cromley (4) found that a temperature of 150° maintained for 30 minutes destroyed 94.5 to 99.9 per cent of the organisms present, and Fay and Olson (5) reported similar results.

The ice-cream industry has been satisfied to use a pasteurizing temperature of 145° F. for 30 minutes because of the low cost of fuel as compared to that when higher temperatures are used, and because it was believed that higher temperatures would injure certain qualities of the ice-cream mix. So fearful of excessive heat were some manufacturers, that they practiced cooling the mix from the pasteurizing temperature to 110° or 120° before passing it through the homogenizer or viscolizer, as otherwise some of the mix would be held at the pasteurizing temperature for a period of one to one and one-half hours, depending on the size of the batch and the capacity of the equipment used. The homogenization of the mix at the lower temperatures resulted in greatly increased viscosity unless the homogenizing pressure was reduced. This high viscosity was desired, since the manufacturer believed that the mix would not whip properly unless it had a fairly high viscosity.

Late years have brought changes in the temperatures employed for pasteurizing and homogenizing. Instead of the temperatures being reduced after pasteurizing and before homogenizing, the mix is usually passed through the homogenizer at the pasteurizing temperature. This practice enables the manufacturer to use higher homogenizing pressures without the danger of obtaining an excessive viscosity in the mix.

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City ordinances and public health officials are calling for greater bacteria-killing efficiency and safety in dairy products. Since higher temperatures do not injure ice-cream mixes in the way that they injure milk, and since ice-cream mixes are composed of numerous products, both dairy and others, it has been found desirable to use higher temperatures in their pasteurization. Some ordinances are known which require temperatures of 145°, 150°, and 160° F. for 30 minutes.

The prejudice against high temperatures has lessened until now we find higher temperatures are advocated for reasons which were not considered formerly. Hening (7), using temperatures of 145°, 155°, and 165° F. for 30 minutes and 180° for 10 minutes, found that the higher temperatures decreased the viscosity of mixes and the size of the fat clumps and improved the whipping properties. The mixes that were pasteurized and homogenized at 180° showed the lowest viscosity, the greatest overrun, and the fewest and smallest fat clumps. The flavor of this ice cream had a cooked aftertaste, while the flavor of the mixes pasteurized at lower temperatures did not. Hening stated that—

The temperature of 180° F. is impractical, but it changes the properties of the mix to the greatest extent. If the temperature of 180° F. was the only one at which these properties of the mix were altered it would show a possibility of the change being due to calcium precipitation.

Martin, Swope, and Knapp (8), using temperatures of 145°, 155°, and 165° F., and holding periods ranging from 15 minutes to 2 hours, found that the overrun and the length of time required for freezing were practically the same in all trials and that no temperature or holding period was definitely superior to any other. They concluded that the quality of the finished ice cream was slightly better at "the lower" temperature of pasteurization. They also found that the acidity and hydrogen-ion concentration of the mixes remained practically unchanged after the mixes had aged for 48 and 72 hours at 40° F.

Olson (9) stated that, other factors remaining the same, the texture and whipping properties of the mix were much improved by higher temperatures, and the viscosity was reduced. When the temperature went much above 150° F., however, a candy flavor developed.

Dahle, Keith, and McCullough (2) used pasteurization temperatures of 150° F. for 30 minutes, 160° for 20 minutes, and 170° for 10 minutes and found that the higher temperatures of pasteurization and homogenization produced mixes lower in viscosity than did the lower temperatures. The mixes pasteurized at the higher temperatures also whipped faster.

#### SCOPE OF THE INVESTIGATION

There are few data available that show the effect of pasteurization and homogenization temperatures on the physical properties of ice-cream mixes. All effects resulting from high temperatures have usually been attributed to pasteurization temperatures. One of the objects of the present study was to determine the effect of the temperature at which the mix is homogenized on its physical properties, as preliminary work had indicated that the homogenization temperature was responsible for some of the results obtained.

Another object of the study was to devise some means of utilizing high temperatures and at the same time overcoming the apparent disadvantage of holding mixes at the high temperature for long periods during homogenization.

## METHODS

Two mixes of different composition were used. One was calculated to test 12 per cent fat, 10.5 per cent serum solids, 15 per cent sugar, 0.36 per cent gelatin, and 37.86 per cent total solids; the other to test 10.5 per cent fat, 12 per cent serum solids, 15 per cent sugar, 0.36 per cent gelatin, and 37.86 per cent total solids.

The mixes were made for the most part from cream, condensed skim milk, skim milk, sugar, and gelatin. In some cases dry skim milk was used, and where a study was made of the effect of pasteurizing and homogenizing temperatures on mixes containing butter, this product was also used. The control mixes always contained cream, condensed skim milk, skim milk, sugar, and gelatin.

Three pasteurizing temperatures were used throughout the experiment, 150° F. for 30 minutes, 170° for 10 minutes, and 180° for 1 minute. Each mix except that pasteurized at 150° was divided into two parts before homogenizing, for the purpose of studying the effect of homogenizing temperatures. One part was homogenized at the pasteurizing temperature and the other part was cooled from 170° or 180° to 150° before homogenizing; in this way the effect of homogenizing temperatures was studied.

Two systems of pasteurizing were used, the vat or holding method and the continuous method. In the holding method the mixes were heated in 10-gallon milk cans. Enough mix was used to provide 5 gallons for each freezing. In the continuous method the ingredients of the mix were added to a 250-gallon glass enameled pasteurizer and heated to 150° F., and the sample to be homogenized at 150° was then removed and held at that temperature for 30 minutes. The mixes heated to higher temperatures were passed through a centrifugal heater which raised the temperature to 170° or 180° as desired just before the mix passed through the homogenizer. The mix remained at these high temperatures only momentarily as it was passed over a surface cooler directly from the homogenizer. One-half of the batches heated to 170° and 180° were drawn off and cooled to 150°, as mentioned before. Table 1 shows the processing temperatures used.

TABLE 1.—*Temperatures used in pasteurizing and homogenizing ice-cream mixes by different processes*

Process No.	Pasteurizing temperature	Time maintained	Homogenizing temperature
	° F.	Minutes	° F.
1	150	30	150
2	170	10	170
3	170	10	150
4	180	0	180
5	180	0	150

For studying the freezing conditions, a 40-quart horizontal brine freezer was used. A preliminary batch of ice cream was frozen before the experimental batches were frozen in order to insure a constant freezer temperature for all batches. The brine temperature was kept as nearly constant as possible during the freezing of each series. The brine was shut off the freezer when a definite consistency in the ice cream was obtained. This consistency was determined by means of an indicating ammeter device and by observation. Over-run and temperature readings were made at the end of each minute. A Fahrenheit thermometer graduated to 0.1 degree was used.

Samples of mix were taken immediately after the cooling operation, and laboratory determinations were made on these samples after 4 hours of aging and after 24 hours of aging. Viscosity determinations were made with the MacMichael viscosimeter at 20° C., using a 3 cm. bob and a No. 28 wire calibrated with a standard sucrose solution. Hydrogen-ion determinations were made by means of a Leeds and Northrup type K potentiometer, using the quinhydrone electrode at exactly 25° C. Titratable acidity determinations were made with Mann's acid test, using N/10 sodium hydroxide with phenolphthalein as an indicator.

Protein-stability determinations were made on 5 c. c. samples of mix to which 10 c. c. of a mixture of 95 per cent alcohol and distilled water were added to produce the first positive signs of feathering or coagulating. The number of cubic centimeters of alcohol in the 10 c. c. sample of alcohol and water was used to indicate the stability of the proteins. The greater this number, the greater is the stability of the proteins.

A Du Nuoy tensiometer was used in determining surface tension. The samples of mix were tempered in a constant temperature water bath to 20° C. Three readings were taken on each mix and the average recorded.

To determine the degree of fat clumping, a hanging-drop slide was made of mix diluted 1 to 300. After examination under the low and high powers of the microscope, the degree of clumping was recorded in numbers, as follows: 0 = no clumping, 1 = evident clumping, 2 = very evident clumping, 3 = prominent clumping, 4 = very prominent clumping, 5 = pronounced clumping, 6 = very pronounced clumping.

The results of the work recorded represent averages of at least three separate trials with every mix used. In some cases averages of many more trials are recorded. A total of 145 mixes was prepared, pasteurized, frozen, and tested in various ways. Twenty-six mixes were prepared and pasteurized and homogenized at each temperature and 15 additional control mixes were made.

#### EFFECT OF THE PROCESSING TEMPERATURES ON SOME PROPERTIES OF 12 PER CENT FAT MIXES WHEN THE VAT METHOD OF HEATING WAS USED

Eight series of mixes containing 12 per cent fat, 10.5 per cent serum solids, 15 per cent sugar, 0.36 per cent gelatin, and 37.86 per cent total solids were made and pasteurized by the vat or holding method of pasteurization. The source of butterfat was cream in four mixes, and unsalted butter in four mixes. The source of additional serum solids was divided equally between skim-milk powder and condensed skim milk.

The effect of processing temperatures on the viscosity of the mixes and fat clumping is plainly shown in Table 2. There it may be seen that the higher the temperature used the lower was the viscosity and the tendency to clump. When the mixes were homogenized at 170° and 180° F. the viscosity was at a minimum. When the mixes were cooled from these temperatures to 150° before homogenizing, the viscosity was greater, showing the importance of homogenizing at the higher temperatures when the mix is pasteurized at these temperatures. The viscosity and fat clumping were greatest in the mixes that were both pasteurized and homogenized at 150°. The degree of fat clumping followed the viscosity closely. The higher temperatures caused a very marked reduction in the fat clumps, except where the temperature was reduced to 150° for homogenization.

TABLE 2.—*Effect of processing temperatures on physical, chemical, and freezing properties of 12 per cent fat mixes when using the vat method of heating*

[Average of eight trials]

Process No. ....	1	2	3	4	5
Pasteurizing temperature, ° F. ....	150	170	170	180	180
Homogenizing temperature, ° F. ....	150	170	150	180	150
Viscosity, centipoises .....	236.98	91.76	173.42	75.80	173.10
Titratable acidity, c. c. N/10 NaOH .....	125	.168	.173	.167	.165
pH .....	6.33	6.33	6.33	6.35	6.34
Fat clumping <sup>a</sup> .....	4	.87	2.8	.37	2.37
Surface tension, dynes. ....	46.25	46.35	46.38	46.41	46.36
Protein stability, c. c. alcohol .....	5.17	5.4	5.45	5.57	5.47
Time to obtain 100 per cent overrun, minutes .....	8.26	7.40	7.85	7.23	7.62
Total score <sup>b</sup> .....	72.31	72.16	72.20	72.33	72.27

<sup>a</sup> 0 = None, 6 = most.

<sup>b</sup> Perfect = 80. Average of scoring by 3 judges.

In this series of trials no particular difference was noted in the surface tension or protein stability due to different temperatures of heating.

The lower temperatures of pasteurizing and also of homogenizing caused an increase in the time required to obtain 100 per cent overrun. While it is not always possible to show a relationship between the whipping ability of a mix and viscosity, it is commonly found that the mixes having the highest viscosity, especially when this is due to fat clumping, whip most slowly. This was generally true in this experiment.

If the results at the highest temperatures are compared, it will be seen that there was some difference between those at 170° and 180° F. when the mix was both pasteurized and homogenized at these temperatures. The higher temperature reduced viscosity and fat clumping more than did the lower. From the standpoint of protein stability and surface tension there was very little difference noted in the results at 170° and 180°.

Practically no difference was found in the score of the different batches of ice cream.

#### EFFECT OF THE PROCESSING TEMPERATURES ON SOME PROPERTIES OF 12 PER CENT FAT MIXES WHEN THE CONTINUOUS METHOD OF HEATING WAS USED

In this experiment the mix was prepared in a 250-gallon pasteurizer. When the mix had reached a temperature of 150° F. it was run through the continuous pasteurizer at the desired temperature and discharged



into a small tank before it passed through the homogenizer. The mix that was cooled from 170° and 180° to 150° before homogenizing was removed from this tank and cooled in 10-gallon milk cans to 150° and then passed through the homogenizer.

Five trials with mixes testing 12 per cent fat and 37.86 per cent total solids were made. The average of these trials appears in Table 3. Three of the mixes contained butter and two contained cream as a source of butterfat. Condensed skim milk furnished the extra serum solids in all mixes except one, in which powdered skim milk was used.

TABLE 3.—Effect of processing temperatures on physical, chemical, and freezing properties of 12 per cent fat mixes when using continuous method of heating

[Average of five trials]

Process No.	1	2	3	4	5
Pasteurizing temperature, ° F.	150	170	170	180	180
Homogenizing temperature, ° F.	150	170	150	180	150
Viscosity, centipoises	2,282.4	98.76	181.64	59.35	340.79
Titratable acidity, c. c. N/10 NaOH	.164	.17	.166	.17	.164
pH	6.30	6.30	6.30	6.31	6.31
Fat clumping <sup>a</sup>	4.6	.6	3.4	.1	2.8
Surface tension, dynes	48.04	46.46	46.01	46.05	46.33
Protein stability, c. c. alcohol	3.48	5.6	5.08	5.6	5.04
Time to obtain 100 per cent overrun, minutes	11.77	9.30	9.66	8.8	10.82
Total score <sup>b</sup>	73.51	72.24	71.21	72.31	71.21

<sup>a</sup> 0 = none, 6 = most.

<sup>b</sup> Perfect = 80. Average of scorings by 3 judges.

The mixes in which butter was used exhibited very high viscosities except in cases where the higher temperatures were employed. The higher pasteurizing temperatures considerably reduced the viscosity, as did also the combination of high pasteurizing and high homogenizing temperatures. This is particularly apparent when process 4 is compared with process 1. For some reason process 5 did not show the reduction in viscosity that appeared in process 3. Process 4 showed the least clumping, and process 2 was next. Although process 5 exhibited a greater viscosity than 3, the fat-clumping tendency was considerably less. In this instance the viscosity and fat clumping did not show the usual relationship.

The high viscosity noted in process 1 had its effect on the freezing of the mixes. The mixes of high viscosity froze more slowly than those of lower viscosity.

This experiment shows conclusively that pasteurizing and homogenizing temperatures of 170° and 180° F. save considerable time in freezing. The difference between processes 1 and 4 was nearly three minutes. For some reason process 5, which had the advantage of a high pasteurizing temperature, did not produce as good freezing results as process 3, in which the mix was heated at a lower temperature but homogenized at the same temperature (150°).

The stability of the protein in these trials varied with the temperatures used. The protein was considerably more stable in the mixes heated to 170° and 180° F., particularly when the mixes were homogenized at these temperatures. Since Doan (3) has shown that there is an inverse relationship between fat clumping and protein stability, this phenomenon was expected, although it was not pronounced in the experiment recorded in Table 2.

# EFFECT OF THE PROCESSING TEMPERATURES ON SOME PROPERTIES OF 10.5 PER CENT FAT MIXES WHEN THE VAT METHOD OF HEATING WAS USED

It has been clearly demonstrated that the higher temperatures of pasteurization and particularly of homogenization reduce fat clumping considerably. Since 10.5 per cent fat mixes do not form fat clumps as readily as 12 per cent fat mixes, it was thought that possibly the high temperatures would not affect these mixes to the same extent. Eight series of mixes testing 10.5 per cent fat and containing the same total solids content as the 12 per cent mixes were studied. One-half of these mixes contained butter and the other half contained cream.

Fat clumping was considerably less prominent in these mixes than in the 12 per cent mixes, which no doubt had much to do with the lower viscosity obtained. While clumping was plainly evident in these mixes, it was more pronounced in the mixes heated at the lower temperatures. The high temperatures caused a pronounced reduction of clumps, but again the most notable reduction was obtained in the mixes which were pasteurized and also homogenized at the higher temperatures. A temperature of 180° F. produced the greatest reduction of fat clumps.

It will be observed in Table 4 that the viscosity of these mixes was generally lower than that of the 12 per cent mixes, and that the higher temperatures to which the mixes were subjected reduced the viscosity. The importance of homogenizing at the higher temperatures may be noted again.

TABLE 4.—*Effect of processing temperatures on physical, chemical, and freezing properties of 10.5 per cent fat mixes, when using the vat method of heating*

[Average of eight trials]

Process No.	1	2	3	4	5
Pasteurizing temperature, °F.	150	170	170	180	180
Homogenizing temperature, °F.	150	170	150	180	150
Viscosity, centipoises	143.06	77.72	106.61	72.86	107.24
Titratable acidity, c. c. N/10 NaOH.	.196	.181	.186	.186	.181
pH.	6.31	6.30	6.30	6.31	6.31
Fat clumping <sup>a</sup>	2.37	.87	2.12	.625	1.88
Surface tension, dynes.	46.85	46.46	46.99	46.73	46.87
Protein stability, c. c. alcohol.	6.0	6.1	5.85	5.8	5.95
Time to obtain 100 per cent overrun, minutes.	8.9	8.5	8.61	8.16	8.7
Total score <sup>b</sup>	73.11	73.02	73.04	73.05	73.11

<sup>a</sup> 0 = none, 6 = most.

<sup>b</sup> Perfect = 80. Average of scorings by 3 judges.

No striking difference was found in the protein stability as a result of the high temperatures. A great difference was not expected, if fat clumping plays an important rôle in protein stability, as the fat clumping in these mixes was not particularly prominent.

While the highest temperatures, 170° and 180° F., produced the overrun in the shortest time, the difference between the longest and shortest time was only about three-fourths of a minute. The difference in the 12 per cent fat mixes was more noticeable.

The data presented in Table 4 indicate that the beneficial effects of high pasteurizing and homogenizing temperatures are not so pronounced in the mixes of lower fat content as in these of higher fat content. If the time saved in freezing the mix is always as slight as

it was in this case, it would not be advisable to pasteurize a mix of this composition above 150° F. because of the cost of additional heat and cooling units.

# EFFECT OF THE PROCESSING TEMPERATURES ON SOME PROPERTIES OF 10.5 PER CENT FAT MIXES WHEN THE CONTINUOUS METHOD OF HEATING WAS USED

Five series of 10.5 per cent fat mixes were represented in this experiment. Three of the series contained butter and two contained cream as the source of fat. One mix contained dry skim milk as a source of extra serum solids, while the remaining mixes contained condensed skim milk. Again the highest temperatures caused reduction in viscosity, but the mixes heated to 180° F. and cooled to 150° before homogenizing were higher in viscosity than the mixes heated to 170° and cooled to 150° before homogenizing. Similar results were also obtained with the 12 per cent fat mixes pasteurized by the continuous method. (Table 5.)

TABLE 5.—*Effect of pasteurizing and homogenizing temperatures on physical, chemical, and freezing properties of 10.5 per cent fat mixes when using the continuous method of heating*

[Average of five trials]

Process No. ....	1	2	3	4	5
Pasteurizing temperature, °F. ....	150	170	170	180	180
Homogenizing temperature, °F. ....	150	170	150	180	150
Viscosity, centipoises. ....	600.89	121.26	126.19	75.85	134.49
Titrate acidity, c. c. N/10 NaOH. ....	.17	.176	.185	.192	.185
pH. ....	6.24	6.26	6.26	6.28	6.27
Fat clumping <sup>a</sup> . ....	3.6	.2	1.6	.2	1.8
Surface tension dynes. ....	48.49	47.02	46.54	46.19	46.61
Protein stability, c. c. alcohol. ....	4.84	6.0	5.64	5.72	5.68
Time to obtain 100 per cent overrun, minutes. ....	9.27	7.08	6.92	6.89	8.09
Total score <sup>b</sup> . ....	72.78	70.99	71.28	71.16	70.98

<sup>a</sup> 0 = none, 6 = most.

<sup>b</sup> Perfect = 80. Average of scorings by 3 judges.

A noticeable reduction in surface tension due to high temperatures was obtained in this series. Little difference has been noted in surface tension previously, except when the mixes were pasteurized by the continuous method.

The degree of fat clumping was practically in the same order as that noted in Table 4, except that 170° F. produced the same amount of clumping reduction as 180°. When the mixes were pasteurized at the higher temperatures and cooled back to 150° for homogenization, the mixes heated to 170° produced greater reduction of clumping than the ones heated to 180°.

The stability of the proteins followed about the same order in this series as in the series pasteurized by the vat method.

An improvement in the overrun was noted in this series as compared to the overrun in the previous 10.5 per cent series heated by the vat method. The mixes heated to 150° F. were more viscous than in the former series, and the reduction in viscosity caused by the higher temperatures was greater in the case of the mixes pasteurized by the continuous system, which may account for the improvement in overrun in this experiment.

While it has been stated before that there is no direct relationship between viscosity and overrun, it appears that the relationship is

quite definite if the viscosity is due largely to fat clumping. When a mix exhibits a high degree of clumping and viscosity, destruction or partial reduction of the clumps and viscosity by high homogenizing temperatures tends to speed up the overrun. In the experiment recorded in Table 5, the saving in freezing time due to the use of high temperatures was 25 per cent, which is considerable. The viscosity of the 150° F. mixes in Table 5, while greater than the corresponding mixes in Table 4, was also greater than some of the 12 per cent fat mixes. This mix showed that the pronounced fat clumping was the cause of the high viscosity, but why this mix should have exhibited greater clumping than mix No. 1 of Table 4 is not known. Similar results have been observed in previous studies on fat clumping.

#### EFFECT OF THE PROCESSING TEMPERATURES ON THE PROPERTIES OF MIXES MADE FROM BUTTER

It has been observed (1) that mixes containing butter exhibit a greater tendency to clump than mixes made from cream. With the increased clumping there is usually increased viscosity and also a tendency to slower freezing. Whitaker (10) suggested that the inferior whipping ability of mixes deriving their fat from butter may be due to the lecithin deficiency of these mixes. Lecithin obtained from the soybean has been used by one of the authors in amounts ranging from 0.1 to 0.4 per cent of the mix without any beneficial results so far as overrun is concerned.

The degree of clumping in mixes deriving their fat from butter may be reduced considerably by the use of high temperatures. The greatest reduction of clumping and the greatest saving in freezing time of mixes containing butter have been obtained in the trials in which the continuous system of heating was used. Table 6 indicates the saving in freezing time because of the high temperatures used.

TABLE 6.—*Effect of processing temperatures on properties of 12 per cent mixes containing butter as the source of fat and made by the vat method of heating*

[Average of four trials]

Process No. ....	1	2	3	4	5
Pasteurizing temperature, ° F. ....	150	170	170	180	180
Homogenizing temperature, ° F. ....	150	170	150	180	150
Viscosity, centipoises .....	373.08	133.30	262.85	82.25	238.4
Titratable acidity, c. c. N/10 NaOH .....	.18	.17	.177	.167	.165
pH .....	6.31	6.30	6.30	6.33	6.31
Fat clumping <sup>a</sup> .....	4.5	1.2	2.5	.5	2.2
Surface tension, dynes .....	45.95	46.39	46.50	46.23	46.03
Protein stability, c. c. alcohol .....	4.4	4.9	4.8	5.1	4.8
Time to obtain 100 per cent overrun, minutes .....	8.69	7.74	8.28	7.3	8.12
Total score <sup>b</sup> .....	70.74	70.54	70.60	70.62	70.66

<sup>a</sup> 0 = none, 6 = most.

<sup>b</sup> Perfect = 80. Average of scorings by 3 judges.

It will be observed that the temperature of 170° and 180° F. when used for both homogenizing and pasteurizing reduced the degree of clumping and the viscosity and speeded up the freezing operation. The results listed in Table 6 were obtained from mixes pasteurized by the vat system of heating, and indicate that when the butter mixes are processed at higher temperatures a substantial saving in time is effected. In another series of trials in which butter mixes were processed by the continuous method of heating, they froze considerably

more slowly. The gain in time due to processing at the higher temperature was considerable, but because of the extremely slow freezing time of the butter mixes processed at 150° the gain was not sufficient to permit the mixes to be frozen as rapidly as previous mixes. Nevertheless, the high temperatures reduced the freezing time four and one-half minutes, as may be seen in Table 7.

TABLE 7.—Effect of processing temperatures on properties of 12 per cent mixes containing butter as the source of fat and made by the continuous method of heating

[Average of three trials]

Process No.	1	2	3	4	5
Pasteurizing temperature, °F	150	170	170	180	180
Homogenizing temperature, °F	150	170	150	180	150
Viscosity, centipoises	3,667.5	87.83	156.3	50.78	333.2
Titratable acidity, c. c. N 10 NaOH	147	16	155	16	155
pH	6.28	6.19	6.29	6.31	6.29
Fat clumping <sup>a</sup>	5.3	3	2.3	0	2.3
Surface tension, dynes	44.71	46.94	46.66	46.54	46.81
Protein stability, c. c. alcohol	3.06	5.7	5.06	5.6	5.06
Time to obtain 100 per cent overrun, minutes	14.33	10.77	11.33	10.03	13.16
Total score <sup>b</sup>	70.74	71.3	89.58	71.17	69.97

<sup>a</sup> 0 = none, 6 = most.

<sup>b</sup> Perfect = 80. Average of scoring by 3 judges.

The tremendous viscosity in process 1 no doubt accounted for some of the delay in freezing. The fat clumping was greatest in this mix when compared to the other mixes containing butter, but was no greater than has been noted in some other mixes. All of the butter mixes were very slow to freeze. Neither clumping nor viscosity could account for this in all cases, as the mixes processed at the high temperatures were practically without clumps and the viscosity was quite low. The acidity, freezing conditions, and all other factors that might have affected overrun were apparent satisfactory. Similar cases are often found where mixes for some unaccountable reason are slow to freeze and whip.

The main object of the experiment was to determine the effect of processing temperatures, and the results obtained demonstrate that freezing time is saved by using high processing temperatures, particularly for homogenizing.

TABLE 8.—Effect of the processing temperature on time required to obtain a 100 per cent overrun in 12 and 10.5 per cent mixes made from cream and butter

		12 per cent mixes					10.5 per cent mixes				
Pasteurizing temperature, °F	150	170	170	180	180		150	170	170	180	180
Homogenizing temperature, °F	150	170	150	180	150		150	170	150	180	150
Method of heating	Source of fat	Minutes required to obtain 100 per cent overrun									
Continuous	Cream	7.44	7.09	7.15	6.95	7.31	7.33	6.75	7.25	7.88	7.43
	Butter	14.33	10.77	11.33	10.03	13.16	10.50	7.30	6.70	6.56	8.50
Vat	Cream	7.83	7.06	7.43	7.16	7.11	8.54	8.46	8.02	7.46	7.78
	Butter	8.69	7.74	8.28	7.30	8.12	9.21	8.55	9.23	8.87	9.63
Average of cream mixes		7.63	7.07	7.29	7.05	7.21	7.93	7.60	7.63	7.67	7.60
Average of butter mixes		11.51	9.25	9.80	8.66	10.18	9.85	7.66	7.66	7.91	9.66

A comparison of the time required to obtain 100 per cent overrun of cream and butter mixes by the two methods of heating is shown in Table 8. An average of all of the 12 per cent cream and butter mixes frozen is also listed. It will be seen that the mixes containing butter yielded the desired overrun more slowly than those containing cream. The high processing temperatures caused a greater reduction of freezing time in the mixes containing butter as a source of fat than in the mixes containing cream. No doubt the reduction of viscosity and clumping was responsible for the saving in freezing time.

Processing the 10.5 per cent mixes containing butter at high temperatures produced results similar to those produced in the 12 per cent mixes. The viscosity of the 10.5 per cent mixes was lower than that of the 12 per cent mixes in most cases, however.

When the overrun of the 10.5 per cent butter mixes is considered, it is noted that high processing temperatures reduce the time needed to obtain the desired overrun in mixes made from butter. A saving of nearly four minutes may be observed in Table 8. This saving occurred in the series of mixes pasteurized by the continuous method; by the vat method the saving in time was approximately one-half minute. At the higher temperatures of processing the difference in freezing time between mixes made from cream and those made from butter was slight.

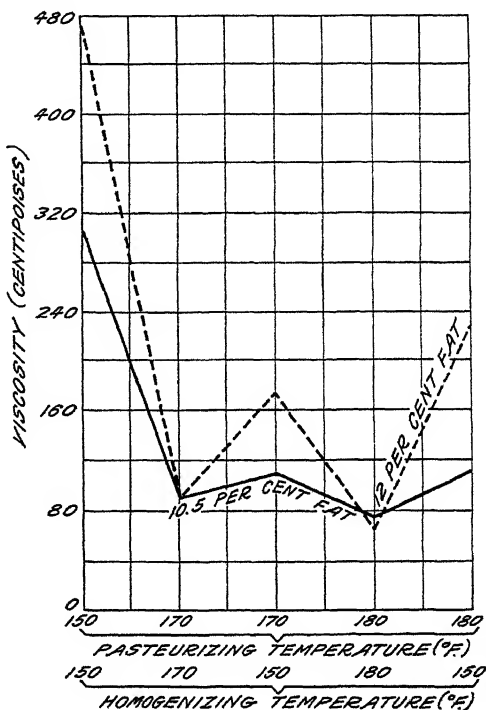


FIGURE 1.—Effect of processing temperatures upon the viscosity of 10.5 and 12 per cent fat mixes

#### COMPARISON OF THE EFFECTS OF HIGH PASTEURIZING AND HIGH HOMOGENIZING TEMPERATURES

As stated earlier, one of the objects of this study was to determine whether the beneficial results of high processing temperatures were due to the pasteurizing or homogenizing temperatures or to a combination of the two. From the data presented in the previous tables it is plain that high processing temperatures were beneficial, the greatest benefits being obtained when the mixes were passed through the homogenizer at the highest temperatures. It does not necessarily follow that the homogenizing temperatures were entirely responsible for these results. The individual effects of the two processes may be plainly seen in Figures 1 to 4, where viscosity, fat

clumping, speed of freezing, and protein stability are considered. These figures represent an average of all 10.5 and 12 per cent mixes

treated by both the vat and continuous systems, and include over 60 mixes for each composition.

Figure 1 shows that the temperature of heating accounted for the greatest reduction in viscosity. The reduction in viscosity due to high pasteurizing and homogenizing temperatures combined was approximately 410 centipoises in the 12 per cent mix; the pasteurization temperature of 180° F. accounted for a maximum reduction of 305 centipoises, while the homogenizing temperature accounted for a reduction of 105. In the 10.5 per cent mixes the combination of high pasteurizing and homogenizing temperatures brought about a reduction of 240 centipoises, of which the high pasteuriza-

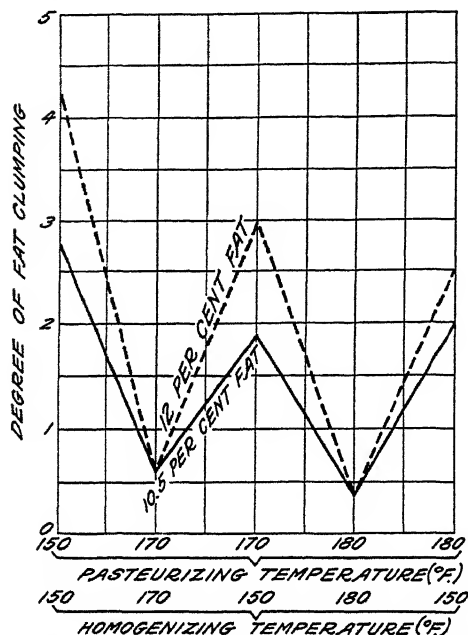


FIGURE 2.—Effect of processing temperatures upon the degree of fat clumping in 10.5 and 12 per cent fat mixes

tion temperature accounted for 198 and the high homogenization temperature for 42.

The temperature of homogenizing was responsible for a greater reduction in fat clumping than the temperature of pasteurization. (Fig. 2.) This seems inconsistent with our knowledge of fat clumping and viscosity. A great deal of the viscosity of mixes is due to fat clumping, and since homogenization temperatures reduced clumping more than did pasteurizing temperatures, it would be expected that homogenization temperatures would affect viscosity more than pasteurization temperatures, but such was not the case, as may be noted. The combination of pasteurizing and homogenizing temperatures reduced the clumping from 4.2 to 0.4, or a total of 3.8 in the 12 per cent mixes. High homogenizing temperatures accounted for a reduction of 2.6, while high pasteurizing temperatures were responsible for a reduction of 1.2. In the 10.5 per cent mixes the reduc-

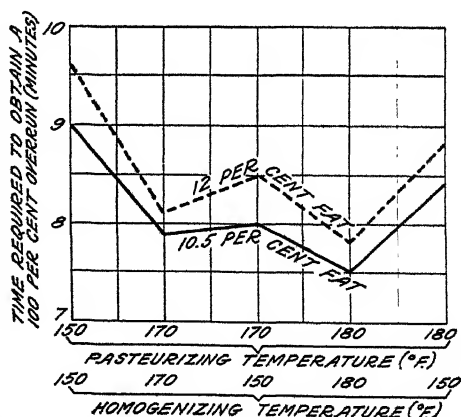


FIGURE 3.—Effect of processing temperatures upon the overrun of 10.5 and 12 per cent fat mixes

tion due to the combination of high pasteurizing and homogenizing temperatures was not as great. This was expected because of the lower fat and higher serum solid content of the mix. The combined reduction was from 2.8 to 0.4, or a total of 2.4, 1.5 being due to the homogenizing temperature and 0.9 to the pasteurizing temperature.

In Figure 3 it will be noted that the reduction in freezing time due to the combined effects of pasteurizing and homogenizing temperature was 1.8 minutes in the 12 per cent mixes, a maximum of 1.1 minutes being due to the pasteurizing temperature and 0.7 of a minute to the homogenizing temperature. A total reduction of 1.4 minutes was obtained in the 10.5 per cent mixes; a reduction of 1.1 minutes being due to the pasteurizing temperature and only 0.3 of a minute to the homogenizing temperature.

Figure 4 shows that protein stability was influenced by the higher temperatures used. The range of difference indicates that the pasteurization temperature was more responsible for the change than the homogenizing temperature. In the 12 per cent mixes the total difference was 1.05 c. c. of alcohol, 0.8 c. c. being due to the pasteurizing temperature and only 0.25 c. c. to the homogenizing temperature. In the 10.5 per cent mixes a smaller difference was noted, as would be expected, since there appears to be an inverse relationship between the degree of fat clumping and protein stability. It will be remembered that the protein stability difference due to high temperatures in the 10.5 per cent mixes was less than in the 12 per cent mixes.

When Figures 1 to 4, inclusive, are examined it will be observed that the mixes pasteurized at 180° F. and homogenized at 150° did not show the beneficial effects that were obtained in the mixes pasteurized at 170° and homogenized at 150° when viscosity and freezing time are considered. Yet the mixes pasteurized and homogenized at 180° benefited more than mixes processed entirely at 170°. Most of this difference occurred in the mixes heated by the continuous system.

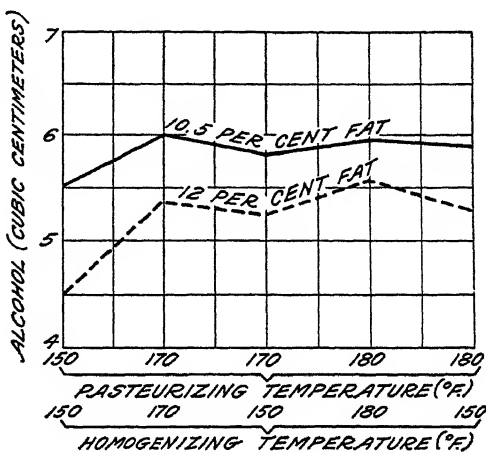


FIGURE 4.—Effect of processing temperatures upon the protein stability of 10.5 and 12 per cent fat mixes

## SUMMARY

The object of the study reported in this paper was to determine the effects of high processing temperatures on certain properties of the ice cream mix, and to study the feasibility of using the flash or continuous system as means of obtaining the high temperatures.

Processing the mix at temperatures of 170° and 180° F. decreases the degree of fat clumping, reduces viscosity and freezing time, and increases protein stability.



The differences obtained between temperatures of 170° and 180° F. is not sufficiently great to warrant using processing temperatures much in excess of 170°.

The greatest benefits to be derived from the use of these high temperatures result when the mix is both pasteurized and homogenized at the high temperatures.

Lowering the temperature of the mix from 170° and 180° F. to 150° F. for homogenizing neutralizes some of the benefits obtained, particularly when the mixes heated to 180° are reduced to 150°.

The continuous system of heating is a simple method for obtaining high temperatures. All of the ingredients may be mixed and heated to 145° or 150° F. in a vat. The temperature can then be raised quickly to 170° or 180° F. by means of a continuous heater just before the mix is passed through the homogenizer.

The high pasteurizing temperature has a greater effect on viscosity, overrun, and protein stability than the high homogenizing temperature, whereas the high homogenizing temperature has a greater effect on fat clumping than the high pasteurizing temperature.

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# A STUDY OF THE COTTON PLANT WITH ESPECIAL REFERENCE TO ITS NITROGEN CONTENT<sup>1</sup>

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## INTRODUCTION

Although cotton has been extensively studied, comparatively little was known regarding its physiology, especially its nitrogen relations, until recently (4, 5, 12, 13, 14, 15, 16).<sup>2</sup> The effect of various conditions of nitrogen fertility upon plants closely spaced—the culture now rather generally practiced under boll-weevil conditions—as compared with those widely spaced, and the stage of growth at which the greatest absorption of nitrogen takes place are important agromomic problems in connection with fertilizer applications and cultural practices. The dry-weight relations of the vegetative parts of the plant, such as leaves, stalks, and roots, as compared with fruits, as well as their nitrogen content, are important in influencing the summation of plant activities expressed in crop yield. This paper presents the results of a study of these factors. The findings here reported were obtained while the writers were engaged in a study of the fruiting habits of the cotton plant, and represent one phase of the investigations carried on at the Pee Dee Experiment Station, Florence, S. C., during the past few years. Data obtained in 1925 to 1927, inclusive, bearing on the dry weight and total nitrogen relations of the various plant parts are presented, as well as the data on the dry weight and total nitrogen of the developing fruit secured in 1928.

## WORK OF OTHER INVESTIGATORS

The chemical analyses of cotton plants made by different workers are not easily compared since there are variations in the percentages of the constituents, evidently due to different varieties, soils, fertilizer practices, and climatic conditions. McBryde and Beal (10), White (20, 21), Blagoveschenski et al. (4, 5), and Ross (18) have found that the percentage of nitrogen varies with the stage of plant development. The analyses by Anderson (1), Fraps (6), McBryde (9), McHargue (11), and Williams (22), made only at one stage of growth, fail to reveal the gradual changes in composition during development although they show differences in the percentage composition of certain elements and compounds as well as appreciable differences in the proportions of stalks, roots, and leaves as compared with seed and lint. The analyses at different stages of growth reported by the above workers undoubtedly account for some, though probably not all, of the variations observed.

Appleton and Helms (2) have studied the rate of absorption of nitrogen by cotton from applications of nitrate of soda and their findings are mentioned in connection with the discussion of data.

<sup>1</sup> Received for publication July 28, 1930; issued May, 1931. Technical contribution No. 2 (new series) from the South Carolina Agricultural Experiment Station.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 702.

Pate (17) made a preliminary report on the rate of absorption of nitrate of soda by cotton.

Maskell and Mason (12, 13, 14, 15, 16) have recently published extensive studies on the transport of various forms of nitrogen in the tissues of the cotton plant.

#### EXPERIMENTAL METHODS

All the plants used in this work were grown from seed of one strain of the Cleveland variety which had been carefully tested and selected for uniformity.

Unless otherwise stated, the samples used in 1925, 1926, and 1927 consisted of eight plants each. The large size attained by the plants toward the end of the season made it almost a physical impossibility, under the conditions that existed, to use more than eight plants in a sample, although a greater number would have been desirable. Two large electric drying ovens and three smaller ones were at times taxed to capacity because of the quantity of material. The aid of six to eight assistants was necessary to clean, separate, and weigh the plant parts in a relatively short time so that all material might be placed in the ovens before an appreciable loss of moisture or considerable enzymatic changes could occur.

Samples were obtained at weekly intervals from the middle of June until the latter part of August in 1925 and 1926. Sampling began with the appearance of flower buds or "squares" and ceased when the plants retained no more young bolls. Four plants carefully selected for uniformity were taken from each of two areas in the plot. In 1927, one plant as it occurred in the row was taken from eight previously selected areas. Thus the personal element in the selection was somewhat lessened. The plants were pulled from the ground about 9 a. m., wrapped in moist cheesecloth and quickly brought to the laboratory, where sand and other foreign particles were brushed from them. Special care was taken to give all contrasted samples identical treatment. Except for a limited time in 1926, when roots also were gathered, only the aerial portions of the plants were used, these being separated into leaves, stalks, and fruits. Both green and dry weights were obtained. After the samples had been dried at 65°-70° C. to avoid carbohydrate decomposition (8), they were coarsely ground and stored in mason fruit jars until analyses could be made. They were then reground to pass an 80-mesh sieve, and 1-gm. or 2-gm. duplicate portions were analyzed by the Kjeldahl method (3) modified to include nitrate nitrogen.

In 1925 and 1926 plats were spaced 6 and 24 inches apart in different rows, but in 1927 and 1928 only a 12-inch spacing was used. In 1925, 1927, and 1928 plants were grown on a Norfolk sandy loam which was known to give the very low yield of less than one-half bale of cotton per acre unless liberal applications of nitrogen, phosphorus, and potassium were made. In 1926 the plants were grown on a soil of the same type which gave the relatively high yield of slightly less than one bale per acre.<sup>3</sup>

Relatively large and small amounts of nitrogen were applied to the plots in the first three years, but only the larger application was made in 1928.

<sup>3</sup>It was impossible to obtain exact yields for these plots since some of the plants were used for chemical analyses.

The plot selected in 1925 contained a very unproductive soil, and it was thought best to use 200 pounds of a 4-8-4 fertilizer per acre on the low-nitrogen plot before planting in order to obtain plants not too seriously retarded in growth. The high-nitrogen plot received 1,080 pounds of the same fertilizer per acre. Subsequent applications of nitrate of soda are indicated in Table 10.

A plot of fairly productive soil was selected in 1926, and in order to get contrasts in plant growth, no fertilizer was added to the low-nitrogen plot, while 1,380 pounds of a 4-8-4 fertilizer per acre were added to the high-nitrogen plot before planting. Subsequent applications of nitrogen are explained in Table 11.

In 1927 the same amounts of phosphorus and potash were added to both plots, only the nitrogen being varied. The amounts applied are shown in Table 12.

### PRESENTATION OF DATA

#### DRY-WEIGHT RELATIONS OF THE PLANT PARTS

The percentages of dry matter in the leaves and stalks of plants grown in 1925 and 1926 under high-nitrogen and low-nitrogen conditions and with 6-inch and 24-inch spacings are given in Tables 1 and 2. There are considerable variations between approximately the same dates in 1925 and 1926, and even from week to week in the same season. There was, however, a distinct tendency for the leaves and stalks of plants from the low-nitrogen plot to be appreciably higher in percentage of dry matter, and therefore less succulent than those of plants from the high-nitrogen plot. This is in accord with facts established for many other plants, in which increased nitrogen is associated with increased succulency. With increasing maturity, plants from both the high-nitrogen and low-nitrogen plots tended to have higher percentages of dry matter. The boll data show the same general trend as those of the leaves and stalks, and so have been omitted.

TABLE 1.—Percentages of dry matter in cotton leaves from plants differently spaced and fertilized, 1925 and 1926

Spacing, plot treatment, <sup>a</sup> and year	Percentage of dry matter in leaves sampled—															
	June 18	June 25	June 29	July 1	July 6	July 8	July 14	July 15	July 21 <sup>b</sup>	July 22	July 28 <sup>b</sup>	July 29	August 4 <sup>b</sup>	August 5	August 11 <sup>b</sup>	August 12
24 inches, HN:																
1925			18.3		18.3		22.0		20.4		22.0		23.6		24.1	31.9
1926	17.5	16.9		16.2		17.8		27.7		22.3		20.7		20.2		22.4
24 inches, LN:																
1925			18.6		18.7		18.0		22.2		23.9		18.2		27.5	26.7
1926	19.3	19.4		18.5		21.4		19.8		25.5		24.2		23.7		24.4
6 inches, HN:																
1925			17.4		17.9		19.9		20.5		24.1		25.1		28.8	
1926	19.9	17.6		15.3		18.7		17.9		21.5		21.7		20.7		21.1
6 inches, LN:																
1925			19.5		21.1		22.2		23.0		28.0		28.0		31.8	29.7
1926	21.1	18.5		17.2		20.7		19.5		23.3		23.3		25.6		25.2

<sup>a</sup> HN=high nitrogen, LN=low nitrogen.

<sup>b</sup> Sample of six plants each.

TABLE 2.—Percentages of dry matter in cotton stalks from plants differently spaced and fertilized, 1925 and 1926

Spacing, plot treatment, <sup>a</sup> and year	Percentage of dry matter in stalks sampled—															
	June 18	June 25	June 29	July 1	July 6	July 8	July 14	July 15	July 21 <sup>b</sup>	July 22	July 28 <sup>b</sup>	July 29	August 4 <sup>b</sup>	August 5	August 11 <sup>b</sup>	August 12
24 inches, HN, 1925	16.4	14.9	14.6	12.8	16.9	17.9	17.9	18.9	20.7	23.3	28.1	28.1	31.1	25.1	28.7	29.3
24 inches, LN, 1925	16.8	16.3	15.7	16.4	20.6	21.2	14.2	21.1	28.1	32.3	34.7	26.8	31.1	29.5	31.1	29.5
6 inches, HN, 1925	17.9	15.0	14.9	12.5	17.8	20.6	24.5	27.0	32.1	34.3	36.6	38.1	33.0	30.4	30.4	30.4
6 inches, LN, 1925	20.4	16.5	12.4	22.9	25.0	22.0	22.5	28.5	42.5	37.6	40.7	36.3	33.7	33.4	33.4	33.4

<sup>a</sup> HN=high nitrogen, LN=low nitrogen<sup>b</sup> Sample of six plants each.

TABLE 3.—Percentage of the total dry weight in each tissue of cotton plants differently spaced and fertilized in 1925

Part of plant, spacing, and plot treatment <sup>a</sup>	Percentage of total dry weight of samples taken—							
	June 29	July 6	July 14	July 21	July 28	August 4	August 11	August 18
Leaves								
24 inches, HN	75.2	65.7	66.1	55.0	52.2	42.1	34.4	32.4
24 inches, LN	70.1	69.1	55.7	56.3	45.2	29.7	29.6	27.9
6 inches, HN	68.7	60.9	54.5	47.4	36.5	30.8	25.7	15.2
6 inches, LN	60.7	51.0	49.7	47.2	34.1	27.2	22.6	15.2
Stalks								
24 inches, HN	24.8	29.5	26.3	31.1	31.8	28.8	23.9	27.2
24 inches, LN	29.9	27.3	35.3	30.7	31.9	32.6	27.4	17.2
6 inches, HN	31.3	34.6	35.1	35.4	29.3	28.6	25.8	23.4
6 inches, LN	39.3	44.0	43.1	36.2	36.7	33.8	37.4	23.4
Squares and bolls								
24 inches, HN	4.7	7.6	13.9	16.0	29.1	41.7	40.4	54.9
24 inches, LN	3.6	9.0	13.0	22.9	37.7	43.0	40.0	61.4
6 inches, HN	4.5	10.4	17.2	34.2	40.6	48.4	40.0	61.4
6 inches, LN	4.9	7.1	16.6	29.1	39.0	40.0	40.0	61.4

<sup>a</sup> HN=high nitrogen, LN=low nitrogen.

TABLE 4.—Percentage of the total dry weight in each tissue of cotton plants differently spaced and fertilized in 1926

Part of plant, spacing, and plot treatment <sup>a</sup>	Percentage of total dry weight of samples taken—										
	June 18	June 25	July 1	July 8	July 15	July 22	July 29	August 5	August 12	August 19	August 26
Leaves:											
24 inches, HN	77.3	73.8	73.3	61.8	64.1	48.5	38.1	36.9	36.4	35.4	25.6
24 inches, LN	78.5	72.1	69.2	61.3	64.0	45.2	39.0	35.4	31.2	27.6	21.2
6 inches, HN	76.5	73.9	70.5	56.4	55.8	43.7	34.8	35.5	30.5	23.0	21.2
6 inches, LN	77.7	71.1	65.5	60.9	52.5	39.9	29.6	31.6	24.9	19.4	18.9
Stalks											
24 inches, HN	22.6	26.2	26.7	34.8	30.2	31.4	28.8	24.3	26.7	29.9	27.0
24 inches, LN	21.5	27.9	30.8	34.5	25.9	33.7	28.2	26.7	21.7	23.5	19.9
6 inches, HN	23.5	26.1	29.5	40.3	38.3	39.8	30.2	28.6	25.3	20.2	20.4
6 inches, LN	22.3	28.9	34.5	35.1	43.2	34.4	32.4	31.6	24.0	22.1	25.8
Squares and bolls:											
24 inches, HN	3.4	5.7	11.2	23.6	30.1	29.3	30.1	29.3	27.5	39.7	53.1
24 inches, LN	4.2	10.1	12.1	24.5	28.4	40.9	42.9	42.9	42.9	53.1	52.6
6 inches, HN	3.3	5.9	8.4	25.7	26.5	37.4	51.0	52.6	52.6	49.4	49.4
6 inches, LN	4.0	4.3	16.8	28.1	27.0	44.8	51.5	51.5	51.5	49.4	49.4
Roots:											
24 inches, HN	8.9	9.4	8.7	7.6	7.2	7.6	7.2	7.6	7.2	7.6	7.6
24 inches, LN	9.0	8.4	9.5	6.1	6.0	5.8	5.8	5.8	5.8	5.8	5.8
6 inches, HN	8.1	9.3	9.4	6.8	5.9	5.8	5.8	5.8	5.8	5.8	5.8
6 inches, LN	8.9	9.9	9.9	6.3	7.0	5.9	5.9	5.9	5.9	5.9	5.9

HN=high nitrogen, LN=low nitrogen.

Tables 3 and 4 show the relative weights of each tissue for 1925 and 1926, under the different conditions of spacing and fertility. The same general trends are evident, although there were differences between the two years, due no doubt to the effect of climatic and soil factors upon the growth and development of the plants. Early in the season prior to fruiting, leaves constituted approximately three-fourths of the dry matter of the plants, whereas only one-fifth to one-third was found in this tissue near the end of the season when the plants had their maximum load of fruit. As the proportion of leaves decreased the proportion of fruit increased steadily, and about two-fifths to three-fifths of the dry matter of the plants was in the fruit near the end of August. The proportion of stalks increased for a time and then gradually declined, although there was no such marked variation in the relative amounts as in leaves and fruits. The roots<sup>4</sup> comprised a relatively small proportion of the weight of the plants, in no case being more than 9.9 per cent. The proportion of roots also declined as the plants matured.

#### NITROGEN RELATIONS OF THE PLANT PARTS

As previously mentioned, all plant samples were analyzed for total nitrogen. Because the results for 1925 were so similar to those for 1926 the 1925 results have been omitted and only those for 1926 and 1927 are presented. The percentage of nitrogen in each dry tissue is given in Tables 5 and 6. The amount of nitrogen per plant has also been calculated, and the percentage of the total present in each tissue is shown in Figures 1, 2, and 3.

Although there are some variations between individual samples, probably due to the use of only eight plants in a sample, the general relations as brought out in the following discussion are quite evident. The percentage of nitrogen in all plant parts was, with a few exceptions, greater under high than under low nitrogen conditions, regardless of the favorable soil and season of 1926 and the relatively unfavorable conditions of 1927.

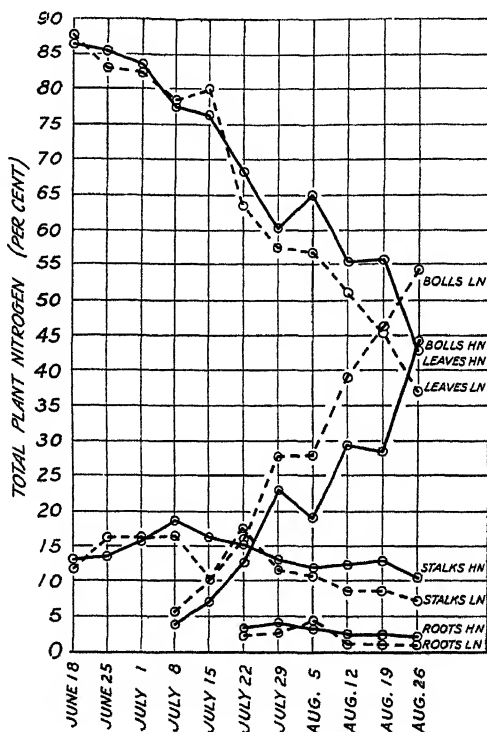


FIGURE 1.—Percentage of total plant nitrogen in leaves, stalks, and bolls of cotton plants grown in high-nitrogen (HN) and low-nitrogen (LN) plots and spaced 24 inches apart in 1926

<sup>4</sup> Only the larger roots were obtained.

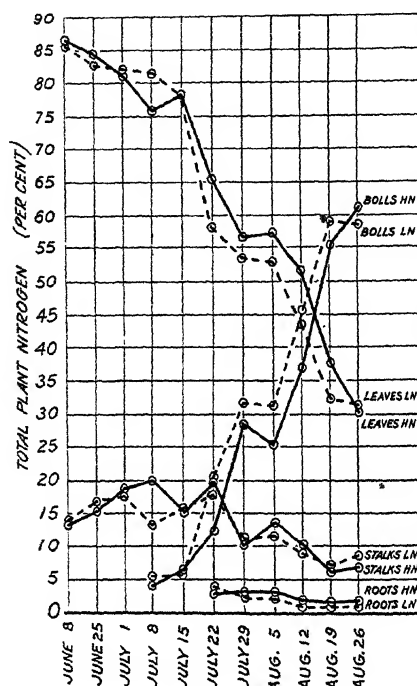


FIGURE 2.—Percentage of total plant nitrogen in leaves, stalks, and bolls of cotton plants grown in high-nitrogen (HN) and low-nitrogen (LN) plots and spaced 6 inches apart in 1926

TABLE 5.—Percentages of nitrogen in the leaves, stalks, squares and bolls, and roots of cotton plants differently spaced and fertilized in 1926

Part of plant, spacing, and plot treatment <sup>a</sup>	Percentage of nitrogen in samples taken—										
	June 8	June 25	July 1	July 8	July 15	July 22	July 29	August 5	August 12	August 19	August 26
<b>Leaves:</b>											
24 inches, HN.....	4.89	4.90	4.75	4.68	4.74	4.25	4.00	4.25	3.97	3.68	3.62
24 inches, LN.....	4.57	4.24	4.02	3.61	4.34	3.53	3.11	3.80	3.26	3.14	3.12
6 inches, HN.....	4.43	4.66	4.88	4.26	4.71	3.76	3.76	4.32	3.97	2.99	2.78
6 inches, LN.....	4.40	4.22	3.84	3.46	3.62	3.52	3.32	2.78	2.82	2.35	2.12
<b>Stalks:</b>											
24 inches, HN.....	2.40	2.38	2.52	2.04	1.74	1.47	1.18	1.30	1.25	1.03	.86
24 inches, LN.....	2.28	2.15	1.76	1.34	1.32		.88	1.01	.74	.66	.63
6 inches, HN.....	2.21	2.41	2.70	1.56	1.33	1.23	.84	1.34	.92	.57	.67
6 inches, LN.....	2.55	2.09	1.60	.98	.88	1.33	.68	.63	.57	.45	.43
<b>Squares and bolls:</b>											
24 inches, HN.....				4.39	4.22	3.61	2.40	2.80	2.60	2.45	2.38
24 inches, LN.....				3.57	3.49	3.27	2.44	2.34	1.91	2.02	1.84
6 inches, HN.....				3.93	3.84	3.62	2.59	2.56	2.28	1.98	2.24
6 inches, LN.....				3.48	3.35	3.19	2.22	1.93	1.46	1.63	1.56
<b>Roots:</b>											
24 inches, HN.....						1.13	1.12	1.01	.82	.74	.61
24 inches, LN.....						.81	.84	1.03	.60	.48	.43
6 inches, HN.....						.93	.88	.95	.76	.48	.58
6 inches, LN.....						1.02	.48	.47	.23	.39	.38

<sup>a</sup> HN=high nitrogen, LN=low nitrogen.

TABLE 6.—Percentage of total nitrogen in the leaves, stalks, and bolls of cotton plants differently spaced and fertilized in 1927

Part of plant, spacing, and plot treatment <sup>a</sup>	Percentage of nitrogen in samples taken—										
	June 16	June 21	June 25	June 28	July 1	July 4	July 7	July 10	July 13	July 17	July 21
Leaves:											
12 inches, HN	4.55	4.16	4.35	4.11	4.23	4.05	4.14	4.69	3.84	3.59	3.33
12 inches, LN	3.31	2.65	2.48	2.55	2.69	3.14	3.32	3.25	3.64	3.07	2.94
Stalks:											
12 inches, HN	2.11	1.87	1.78	1.94	1.76	1.92	1.81	1.75	1.45	1.40	.99
12 inches, LN	1.22	1.06	1.32	1.15	.90	1.20	1.00	.96	1.13	1.06	.88
Bolls:											
12 inches HN					4.25	4.35	4.17	3.98	3.82	3.77	3.11
12 inches, LN								3.19	3.45	2.69	2.67

Part of plant, spacing, and plot treatment <sup>a</sup>	Percentage of nitrogen in samples taken—							
	July 24	July 27	July 30	August 2	August 5	August 8	August 11	August 15
Leaves:								
12 inches, HN	3.58	3.67	3.68	3.74	3.58	3.39	3.66	2.95
12 inches, LN	3.23	3.51	3.47	2.69	3.08	2.98	3.27	3.18
Stalks:								
12 inches, HN	1.12	1.21	1.01	1.16	.88	.95	.86	.74
12 inches, LN	1.03	.97	1.02	.09	.96	.73	.88	.84
Bolls:								
12 inches HN	2.91	2.85	2.86	2.56	2.30	2.29	2.34	2.23
12 inches, LN	2.42	2.39	2.25	1.98	2.12	1.99	1.87	2.01

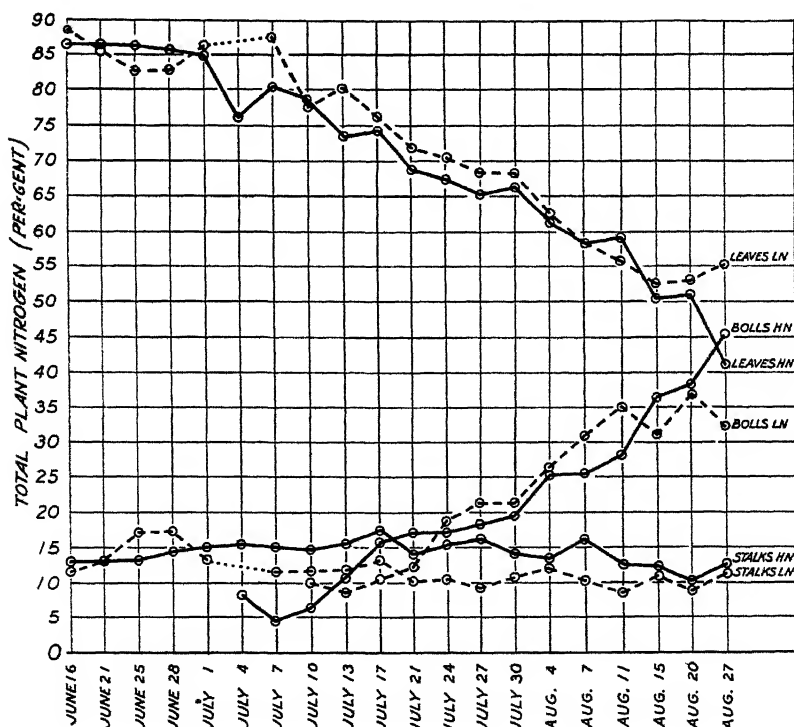
<sup>a</sup> HN=high nitrogen, LN=low nitrogen.

FIGURE 3.—Percentage of total plant nitrogen in leaves, stalk, and bolls of cotton plants grown in high-nitrogen (HN) and low-nitrogen (LN) plots and spaced 12 inches apart in 1927



Anderson (1) by applying nitrogen, phosphorus, or potash to an unproductive soil was able to increase the percentage of that constituent in cotton plants grown on it. White (21), on the other hand, concluded that the relative amounts of nitrogen and mineral ingredients in the plants did not appear to be greatly affected by the quantity supplied. His tables for 1911 and 1912 show, however, that when a fertilizer containing no nitrogen was applied, the percentage of nitrogen in the plants was lower than when a standard fertilizer containing nitrogen was used. Williams (22) reports that cotton plants grown on unfertilized soil had the highest percentage of nitrogen in the leaves and other vegetative parts. This is not in agreement with the findings of Anderson (1) and White (21), nor with those presented in this paper. It can be seen in Table 6 that one analysis of a small number of plants might lead to a conclusion such as that of Williams, since the percentages of nitrogen in leaves on August 15, and stalks on August 5, 11, 20, and 27, were smaller under high-nitrogen conditions. The conception of balance of the nutrients of the soil solution is of importance in an interpretation of such results. Thomas (19, p. 426) states: "However \* \* \* experimental evidence exists to show that the concentration of nutrients of the soil solution may be the factor of greatest influence in determining the course of absorption."

It is evident from Table 5 that widely spaced plants generally show a higher percentage of nitrogen in their tissues than those closely spaced after approximately July 1, when the competitive effects of close spacing become noticeable.

The percentage of nitrogen in the tissues was generally smaller in 1927 than in 1926 even if the 12-inch spacing is compared with the more crowded conditions of the 6-inch spacing. The seasonal factors associated with a large number of cloudy days and many rains were probably of importance in this connection, since conditions in 1927 were very unfavorable for cotton production, although the soil of the plot used was less productive than that employed in 1926 and the fertilizers were also different. The unfavorable effect of the seasonal complex is strongly indicated, however, since the Cleveland 5 variety produced on an average only 1,159 pounds of seed cotton per acre on all other experiment station plots in 1927 as compared with 2,417 pounds in 1926.

An examination of Figures 1, 2, and 3 reveals the fact that the leaves contained from 80 to 90 per cent of the plant's nitrogen when the first few squares were present, and that this proportion steadily declined until 30 to 55 per cent was present the latter part of August, when approximately a full load of fruit had been produced. The load of fruit evidently affected the proportions of the total nitrogen in the leaves of plants grown on both high and low nitrogen plots in 1926 and 1927. With a large crop of fruit in 1926 a relatively smaller percentage of the total amount of nitrogen was present in the leaves of closely spaced plants grown under low-nitrogen than in those grown under high-nitrogen conditions. With a very poor retention of fruit by all plants in 1927, the leaves from plants on low-nitrogen plots had a greater proportion of the total nitrogen than did those from high-nitrogen plots, even though there was a greater evident nitrogen starvation of the plants grown under low-nitrogen conditions.

The fruits from the plants on low-nitrogen plots showed in 1926 approximately 55 per cent of the total nitrogen, whereas in 1927 they contained only 37 per cent. That the cotton fruits made rapid demands for nitrogen is shown by the fact that 40 to 60 per cent of the entire amount of this constituent was found in the fruits the latter part of August, whereas relatively minor proportions were present four or five weeks earlier. Maskell and Mason (12) in their first paper on the transport of nitrogenous substances in the cotton plant infer that nitrogen goes very largely to the bolls and little to the roots. This was found to be true in the studies here reported, as will be shown later. Figures 1 and 2 show that closely spaced plants had an appreciably greater proportion of the nitrogen in the fruits than had the widely spaced plants.

The roots contained the smallest amount of nitrogen, this constituent never exceeding 4.1 per cent of the total and in some instances being as low as 1.0 per cent. As may be seen in Table 5, there were only slight differences in the percentage of nitrogen in the roots of plants from high and low nitrogen plots. In all the plants the proportion of nitrogen in the roots declined appreciably as the plants matured. The stalks also contained a small proportion of the total nitrogen, in some cases as little as 7 per cent, at the time when practically no more fruit buds and bolls were being retained. The bolls and leaves accumulated by far the greatest amount of nitrogen both in total nitrogen and in percentage concentration.

As is shown in Table 5, some very distinct differences in the percentage of nitrogen in composite samples of squares and bolls of plants grown under high-nitrogen and low-nitrogen conditions were observed in the late samples of 1926. It therefore became of interest to know whether these differences also existed in the seed. Williams (22) had found that a high nitrogen content of the seed was associated with the use of fertilizers having a high nitrogen and phosphorus content. Samples of August 19 and August 26, therefore, were divided into boll walls (carpels), lint, and seed, and nitrogen analyses were made. The results are presented in Table 7. It should be remembered that the bolls were of varied and unknown ages, many being almost mature, and that the closely spaced plants of the low-nitrogen plots were more mature than those of the high-nitrogen plots. There were no significant differences in the percentage composition of nitrogen in the seed with the exception of the sample collected August 26 from the close spacing on the high-nitrogen plot. It is possible that the relatively high percentage of nitrogen in the seed of plants from this plot was due to a sampling error. The low percentage of nitrogen in the boll walls and lint of both the widely and closely spaced plants from the low-nitrogen plots is consistent and quite evident. The data of this table seem to indicate that the nitrogen content of the seed was maintained at approximately the same concentrations, even though tissues such as leaves, stalks, boll walls, and lint showed a relatively low nitrogen content with a low supply of nitrogen.

Further studies of the fruits were made in 1928 by analyzing a series of bolls 7, 10, 13, 17, and 21 days of age from plants grown under high-nitrogen and low-nitrogen conditions and spaced 12 inches apart. The data are presented in Tables 8 and 9. There were no signifi-

cant differences in the nitrogen content of entire bolls, the separate walls, or seed and lint during the first 21 days, a period in which the bolls had attained full size but not one-half their maturity. It may be inferred that the differences in composition of the boll walls and lint in 1926 (Table 7) were due to the greater maturity of those samples.

TABLE 7.—Percentages of nitrogen in boll walls, lint, and seed of cotton plants differently spaced and fertilized, 1926

Tissue	Date of sampling	Percentage nitrogen when spacing and plot treatment were as indicated			
		24 inches, HN <sup>a</sup>	24 inches, LN <sup>a</sup>	6 inches, HN <sup>a</sup>	6 inches, LN <sup>a</sup>
Boll wall.....	Aug. 19	2.35	1.75	1.73	1.04
	Aug. 26	2.49	1.40	1.84	.96
Lint.....	Aug. 19	1.17	.70	.73	.38
	Aug. 26	.78	.47	.58	.53
Seed.....	Aug. 19	3.18	3.27	2.97	2.85
	Aug. 26	3.29	3.18	3.51	2.67

<sup>a</sup> HN=high nitrogen, LN=low nitrogen.

TABLE 8.—Percentages of nitrogen in bolls, boll walls, and seed and lint from cotton plants differently spaced and fertilized, 1928

Part of plant	Percentage nitrogen at age in days and with plot treatment indicated									
	7		10		13		17		21	
	HN <sup>a</sup>	LN <sup>a</sup>	HN <sup>a</sup>	LN <sup>a</sup>	HN <sup>a</sup>	LN <sup>a</sup>	HN <sup>a</sup>	LN <sup>a</sup>	HN <sup>a</sup>	LN <sup>a</sup>
Bolls.....	2.50	2.32	2.15	2.10	2.07	2.05	1.95	1.81	1.83	1.80
Boll walls.....	2.36	2.11	2.06	2.00	2.00	1.95	1.86	1.63	1.86	1.69
Seed and lint.....	2.98	3.04	2.37	2.32	2.18	2.25	2.09	2.11	1.80	1.93

<sup>a</sup> HN=high nitrogen, LN=low nitrogen.

TABLE 9.—Proportions of nitrogen in each tissue of bolls from cotton plants differently spaced and fertilized, 1928

Part of plant	Percentage nitrogen at age in days and with plot treatment indicated									
	7		10		13		17		21	
	HN <sup>a</sup>	LN <sup>a</sup>	HN <sup>a</sup>	LN <sup>a</sup>	HN <sup>a</sup>	LN <sup>a</sup>	HN <sup>a</sup>	LN <sup>a</sup>	HN <sup>a</sup>	LN <sup>a</sup>
Boll walls.....	74.3	68.9	64.1	64.2	59.9	63.2	58.4	57.4	47.8	50.5
Seed and lint.....	25.7	31.1	35.9	35.8	40.1	36.8	41.6	42.6	52.2	49.5

<sup>a</sup> HN=high nitrogen, LN=low nitrogen.

Though the different forms of nitrogen in the various tissues are not revealed by the analyses which have been made, yet the data presented give indications that the stalks of plants from low-nitrogen plots and all roots stored relatively little nitrogen, but the leaves and boll walls probably serve as organs for the accumulation of more nitrogen than the minimum concentration required for growth. It may perhaps be assumed that the concentration of nitrogen in the stalks and roots of plants from low-nitrogen plots, in general, is little if at all above the minimum metabolic requirements of these tissues. If, for any reason, the absorption of nitrogen ceases or is retarded, it seems probable that nitrogen present in excess of the minimum requirements in all leaves, boll walls, or stalks of plants from high-nitrogen plots may be used for the further development of the growing seed, which, as has been shown for certain ages at least, tends to

have the same percentage of nitrogen in plants grown under both high-nitrogen and low-nitrogen conditions.

Attention has been called to the relatively small quantity of the total nitrogen present in the cotton stalks. The leaves, on the other hand, contained from two to three times the amount of nitrogen in the stalks. The bolls, and particularly the seed, contained a considerable proportion of the nitrogen of the cotton plant in the latter part of the season. Under conditions of close spacing the seed may contain the bulk of the nitrogen of the crop, the leaves only one-fourth or one-fifth, and the stalks approximately one-tenth.

#### ABSORPTION OF NITROGEN

Information on the rapidity at which the available nitrogen in the soil is removed by cotton plants is a matter of considerable economic importance and should be of value in improving fertilizer practices by possibly reducing the loss of the soluble nitrogenous constituents. Such loss would depend upon the amount of available nitrogen which is subjected to leaching or changed into other forms. The total nitrogen in the plants calculated as pounds per acre of nitrogen is shown in Tables 10, 11, and 12, and when considered in connection with the nitrate applications gives some information as to the periods of greatest absorption of nitrogen from the soil. Certain irregularities occur in these tables, as, for example, the possible loss of nitrogen early in the season. An actual loss of nitrogen may have taken place, though variations in the 8-plant samples seem the more plausible explanation.

TABLE 10.—*Pounds of nitrogen per acre in cotton plants differently spaced and fertilized, and analyzed in 1925*

[Applications of 100 pounds of  $\text{NaNO}_3$  per acre were made on May 23 to all plots, and on June 19 and July 17 to HN plots]

Spacing and plot treatment <sup>a</sup>	Pounds of nitrogen per acre in plants sampled—							
	June 29	July 5	July 12	July 19	July 26	August 2	August 9	August 16
24 inches, HN.....	5.8	19.6	31.2	43.7	58.6	80.2	91.2	79.0
24 inches, LN.....	7.6	12.7	26.0	33.7	61.5	48.0	52.7	57.5
6 inches, HN.....	23.9	43.2	64.0	70.7	87.0	89.0	69.8	53.8
6 inches, LN.....	32.4	27.0	36.1	43.7	40.6	50.7	50.5	41.2

<sup>a</sup> HN=high nitrogen, LN=low nitrogen.

TABLE 11.—*Pounds of nitrogen per acre in cotton plants differently spaced and fertilized, and analyzed in 1926*

[Applications of 75 pounds of  $\text{NaNO}_3$  per acre were made on June 4 to all plots and on July 1 and July 23 to HN plots]

Spacing and plot treatment <sup>a</sup>	Pounds of nitrogen per acre in plants sampled—										
	June 18	June 25	July 1	July 8	July 15	July 22	July 29	August 5	August 12	August 19	August 26
24 inches, HN.....	2.3	3.7	8.9	22.0	32.9	39.3	46.3	81.9	86.3	79.8	69.7
24 inches, LN.....	2.6	6.2	7.7	13.4	20.5	35.7	37.7	52.5	56.7	83.7	64.8
6 inches, HN.....	8.9	13.8	24.5	45.4	80.6	70.1	73.2	83.1	144.4	132.8	194.0
6 inches, LN.....	5.7	12.9	18.4	31.1	35.1	61.7	53.2	56.9	61.8	83.8	65.6

<sup>a</sup> HN=high nitrogen, LN=low nitrogen.

TABLE 12.—Pounds of nitrogen per acre in cotton plants differently spaced and fertilized, and analyzed in 1927

[Applications of 75 pounds per acre of  $\text{NaNO}_3$  were made to the HN plots on June 7, June 30, and July 21]

Spacing and plot treatment <sup>a</sup>	Pounds of nitrogen per acre in plants sampled--										
	June 16	June 21	June 25	June 28	July 1	July 4	July 7	July 10	July 13	July 17	July 21
12 inches, HN-----	5.4	6.1	9.5	11.5	11.7	22.7	22.4	38.0	32.5	43.6	35.2
12 inches, LN-----	2.2	1.8	2.9	3.0	2.5	-----	4.7	5.3	9.0	5.8	7.0

Spacing and plot treatment <sup>a</sup>	Pounds of nitrogen per acre in plants sampled—									
	July 24	July 27	July 30	August 2	August 5	August 8	August 11	August 15	August 20	August 27
12 inches, HN-----	51.4	50.1	58.5	55.4	73.2	80.9	92.3	100.4	77.8	101.3
12 inches, LN-----	8.1	6.6	16.5	12.3	25.5	16.7	13.8	30.6	36.9	38.5

<sup>a</sup> HN=high nitrogen, LN=low nitrogen.

It is seen that closely spaced plants contained larger quantities of nitrogen relatively early in the season than the widely spaced plants. The ordinary agricultural practice in South Carolina is to apply only one side application of 15 to 30 pounds of readily available nitrogen at the time of thinning. In these experiments the time of thinning was May 22. It will be noted in Table 11 that the closely spaced plants did not contain 13.5 pounds of nitrogen per acre, or the equivalent of 75 pounds of nitrogen as nitrate of soda, until June 25, even though the high-nitrogen plots had received 1,380 pounds of 4-8-4 fertilizer per acre at planting on April 20, and a subsequent application of 75 pounds of nitrate of soda per acre on June 4. The total nitrogen applied to the high-nitrogen plots in 1926 was equivalent to 97 pounds of nitrogen per acre. The analyses of widely spaced plants do not account for the total at any time, though twice this amount was found in closely spaced plants on August 26.

In general the closely spaced plants absorbed more nitrogen per acre under high-nitrogen conditions than did the widely spaced plants. However, this does not appear to have been true after August 2 (Table 10) in 1925. The decreasing nitrogen was undoubtedly due to a heavy shedding of leaves which occurred fairly early on account of dry weather and the highly competitive conditions existing between the plants spaced 6 inches apart.

Appleton and Helms (2) grew cotton plants in pots in the greenhouse to the stage of flower-bud production and found large increases in the percentage of nitrogen in the young plants soon after applications of nitrate of soda.

White (20) concluded from analyses of cotton plants at four stages of growth during four seasons that there is considerable regularity in the proportionate intake of nitrogen for each stage of development of the plant. He found that 34 per cent of the nitrogen was taken from the soil in the first period from sprouting to first flower buds, 32 per cent from first flower buds to first blooms, 18 per cent from first blooms to first open bolls, and 16 per cent from first open bolls to the maturity of all bolls. The results presented in this paper for 1926 are

in striking contrast to those of White, since 40 to 50 per cent of the nitrogen present in the plants at the end of fruit setting, but before the maturity of all the fruit, was taken up in the relatively short period of approximately two weeks. An application of nitrate of soda was made on July 23 during a very dry period, but this, as well as an earlier application, was probably not available to the plants until a rain of 2.38 inches fell on July 28. This rainfall occurred during a very active period of growth and fruit production and resulted in the rapid absorption of nitrogen from July 28 to August 12, as is shown in Table 11. This furnishes an excellent example of the possibly beneficial effects to cotton of late applications of nitrogen if the plant growth and seasonal conditions seem to demand it. Plant analyses in connection with a study of the effects of the time of application of nitrogen on final yield might partly explain some of the variable results obtained by Hall and Armstrong (7). The same amount of nitrate of soda as a side application produced the greatest yield in some years when all of it was added at the time of thinning, while in other years divided applications at thinning and the appearance of first squares gave the largest yields.

#### SUMMARY

Cotton plants were grown in field plots in 1925, 1926, and 1927 with a relatively low supply of nitrogen and with a relatively high supply of nitrogen.

The concentration of nitrogen in the leaves, stalks, and fruits was generally greatest where nitrogen was relatively abundant (high-nitrogen plots), and was correlated with a greater succulence of the tissues; or, conversely stated, a greater percentage of dry matter was present in the tissues of plants from plots where the nitrogen was relatively scarce (low-nitrogen plots).

The developing seed and lint from 7 to 21 days of age showed approximately the same nitrogen concentration under high-nitrogen and low-nitrogen conditions. The boll walls and lint at later stages in 1926 showed lower concentrations of nitrogen with the low nitrogen supply.

The relative proportions of the total nitrogen present in the stalks were lower in plants from low-nitrogen plots than in plants from high-nitrogen plots.

The percentage of nitrogen in all tissues except possibly seed tended to decrease with maturity.

In 1925 and 1926 closely spaced plants had a larger proportion of stalks to total dry weight and a larger proportion of their nitrogen in bolls than did widely spaced plants. Moreover, they absorbed a larger proportion of the total nitrogen relatively early and in 1926 took up considerably more nitrogen.

Just prior to the rapid production of flower buds, about three-fourths of the dry weight of the plant and from 80 to 90 per cent of the nitrogen were present in the leaves. The proportion of leaf weight to plant weight steadily declined and the proportion of boll weight to plant weight steadily increased until the end of the fruit-setting season, when only one-fifth to one-third of the dry weight of the plant and from 30 to 55 per cent of its nitrogen was present in the leaves. At this time from 40 to 60 per cent of the nitrogen of the plants was found in the bolls. At 21 days of age when bolls had

reached full size but not maturity, approximately one-half of the boll nitrogen was present in the wall tissue.

Early in the season the stalks showed an increasing proportion of the total weight and later a gradual decline, with a decrease in the proportion of nitrogen, which was as low as 7 per cent of the total on August 26, 1926.

The proportion of the root weight to the total weight was relatively small, ranging from 9.9 to 5.8 per cent during the period of root collections in 1926. The proportion of the total nitrogen was even less, ranging from 4.1 to only 1 per cent.

The total quantity of nitrogen absorbed by the plants early in the season was not large, although the rate of absorption was very rapid owing to the increase in size of the small plants. Under certain seasonal conditions, however, such as prevailed in 1926, older plants absorbed from 40 to 50 per cent of the nitrogen of the crop, equivalent to several hundred pounds per acre of nitrogen as nitrate of soda, in about two weeks.

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## PROGRESSIVE CHANGES IN THE WAXLIKE COATING ON THE SURFACE OF THE APPLE DURING GROWTH AND STORAGE<sup>1</sup>

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### INTRODUCTION

In a previous communication one of the writers<sup>2</sup> reported the isolation and identification of the three principal constituents of the ether-soluble waxlike coating present on the surface of the apple. The investigation has now been extended to include a study of the progressive changes in this coating during growth and storage of the fruit. This phase of the work was prompted by questions arising in regard to the influence of the natural waxy coating of the fruit upon (1) the adhesiveness of sprays applied, (2) the removal of spray residues, and (3) the development of storage scald.

It is very probable that the chemical and physical properties of the surface-coating constituents and the relative quantities of these substances play an important rôle in the effectiveness of spray applications. Preliminary studies have shown that the percentage composition of the waxy coating of apples and therefore its physical properties change gradually throughout the growing season. A thorough study of the chemical and physical changes should, therefore, result in the accumulation of data which might be of considerable value in spray-research work.

It is recognized in certain sections of the country that the character of the surface covering of apples is of considerable importance in connection with methods of spray removal. In the Pacific Northwest a large percentage of the harvested apple crop is washed with chemicals to reduce the quantity of arsenic from spray materials to the amount permitted by law. Varieties such as Esopus Spitzenburg and Arkansas Black are more difficult to clean than others,<sup>3</sup> even when treated immediately after picking. Furthermore, any delay in cleaning these and certain other varieties results in increased difficulty in removing most of the spray residue. A possible explanation of this behavior is that waxlike substances may diffuse to the surface, particularly after the fruit is picked, and render the removal of the arsenicals more difficult. This theory is supported by the fact that such varieties feel greasier to the touch after standing and that oil sprays, especially when applied late in the growing season, make the fruit more difficult to clean later.

<sup>1</sup> Received for publication Nov. 29, 1930; issued June, 1931.

<sup>2</sup> SANDO, C. E. CONSTITUENTS OF THE WAX-LIKE COATING ON THE SURFACE OF THE APPLE. *Jour. Biol. Chem.* 56: 457-468. 1923.

<sup>3</sup> DIEHL, H. C., FISHER, D. F., HARTMAN, H., MAGNESS, J. R., and ROBINSON, R. H. REMOVAL OF SPRAY RESIDUE FROM APPLES AND PEARS IN THE PACIFIC NORTHWEST. *U. S. Dept. Agr. Circ.* 59, 20 p. 1929.

It has been repeatedly suggested that there is a relation between the nature of the skin and the keeping quality of an apple. The waxy covering of the skin should be of especial importance in a consideration of the matter of gaseous exchange between the interior of the fruit and the external atmosphere. At the present time storage scald is believed to be caused by gaseous or volatile products that are formed within the fruit as a result of abnormal respiratory processes.<sup>4</sup> Abnormal respiratory conditions may be produced by retarding the escape of carbon dioxide from the fruit and by hindering the entrance of oxygen. These changes might be associated to some extent with differences in permeability of the waxy coating on the surface of the fruit. It is not unreasonable, therefore, to assume that a possible correlation may exist between the physical nature of the apple coating and the development of storage scald.

In view of the fact that previous chemical work now enables us to determine quantitatively the surface constituents of the apple, and because of the importance of a further knowledge of these constituents to the solution of the above-mentioned problems, it seemed desirable to ascertain varietal differences and the effect of the stage of development of the fruit upon the percentage composition of the waxy coating.

#### PREVIOUS CHEMICAL INVESTIGATION OF APPLE-SURFACE COMPOUNDS

From the work mentioned above<sup>5</sup> it is evident that the surface coating may be separated into two distinct fractions, one soluble and the other insoluble in low-boiling petroleum ether. The soluble or low-melting portion is, at ordinary temperatures, a greenish lardaceous mass, becoming semiliquid but of a greasy consistency when rubbed between the fingers. The bulk of this petroleum-ether soluble portion has been shown to consist principally of the hydrocarbon triacontane ( $C_{30}H_{62}$ ), melting at  $63.5^{\circ}$  to  $64^{\circ}$  C., and a much smaller quantity of the alcohol heptacosanol ( $C_{27}H_{56}O$ ), melting at  $81^{\circ}$  to  $81.5^{\circ}$ . Small quantities of unidentified substances of a true oily nature probably are also present. The second fraction, the petroleum-ether insoluble portion, was found to consist of a greenish-yellow powder, resinous to the touch and very water repellent. In the pure crystalline state the compound melted at  $285^{\circ}$ . It was originally designated "malol," but subsequent work by Van der Haar<sup>6</sup> has shown it to be identical with ursolic acid (urson), previously isolated from the leaves of bearberry (*Arctostaphylos uva-ursi*). Further work by one of the writers<sup>7</sup> has confirmed this identification of malol with ursolic acid and the probable correctness of the empirical formula  $C_{30}H_{48}O_3$  previously assigned to it.<sup>8</sup>

#### EXPERIMENTAL MATERIAL

Material for the analytical work herein reported was obtained from three sources: In 1926, from the variety orchard of the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C.; in 1927, from the Arlington Experiment Farm and from Wenatchee, Wash.,

<sup>4</sup> BROOKS, C., COOLEY, J. S., and FISHER, D. F. APPLE-SCALD. Jour. Agr. Research 16: 195-217. 1919.

<sup>5</sup> SANDO, C. E. Op. cit. (See footnote 2.)

<sup>6</sup> VAN DER HAAR, A. W. UNTERSUCHUNGEN ÜBER DIE SAPONINE UND VERWANDTE KÖRPER. XIII. DIE IDENTITÄT VON MALOL MIT URSON (URSOLSÄURE). Rec. Trav. Chim. Pays-Bas 43: 548-549. 1924.

<sup>7</sup> SANDO, C. E. URSOLIC ACID. Jour. Biol. Chem. 90: 477-495. 1931.

<sup>8</sup> SANDO, C. E. Op. cit. (See footnote 2.)

except the McIntosh variety, which came from British Columbia. Apples obtained from the Arlington Experiment Farm were collected at three different stages of growth, designated, respectively, as early, medium, and mature. Material representing the mature stage was also placed in storage at 32° F. and is designated as "storage." The fruit from the Pacific Northwest, representing the mature stage, was picked at the usual harvesting time.<sup>9</sup>

## METHODS OF ANALYSIS

### PREPARATION OF SAMPLES

In order to obtain representative samples at each stage of growth and to avoid the necessity of analyzing a large number of individual fruits to determine existing variations, composite samples were used. Most of these composite samples represented 75 apples, although in certain cases, as indicated in the tables, fewer apples were used.

The method of collecting the fruit was fairly uniform. In most cases one tree supplied sufficient material throughout the entire season for each variety studied. Whenever two trees of the same variety were employed, adjacent ones were selected and approximately equal quantities were removed from each tree. Care was taken to obtain fruit from the outer branches having a southern or southeastern exposure. The apples, regardless of differences in size, were measured with calipers to the nearest millimeter for the purpose of recording individual diameters.

Samples were obtained from each apple by taking, with the aid of a cork borer, one disk each from the sunny and the shady side. In the early stage of some varieties, and even in the mature stage of such fruit as Grimes Golden and Rhode Island Greening, it was difficult to distinguish any marked difference in color between the two sides. In such instances a mixed sample was used. By using the same cork borer throughout the investigation, the disks obtained had a uniform diameter of 21.5 mm. As removed from the apple, the disk consisted of the waxlike coating, the ether-insoluble cuticle, epidermal cells, and adjacent tissues. (Fig. 1, A and B.) After immersing the disk in dilute hydrochloric acid (15 ml. concentrated acid to 2,000 ml. water) from 24 to 48 hours, the thin outer layer was separated from the flesh by means of tweezers. This layer consisted of the cuticle and its waxy coating together with the epidermal cells or a portion of them, to which the subepidermal cells sometimes adhered. (Fig. 1, C.) It was noticed that the cuticle was somewhat more difficult to remove from the sunny side than from the shady side of the same apple, and also that it could not be removed as easily from mature as from immature fruit. The cuticle was so difficult to remove in the described manner from mature fruit in certain cases, especially that of the York Imperial variety, that it was necessary to free it from the adhering tissues by careful scraping with the thumb-nail.

After separation, the cuticle was rinsed with tap water and then allowed to stand overnight in distilled water to insure complete removal of the hydrochloric acid. Each composite sample was then washed and sucked dry on a Büchner funnel and dried further over sulphuric acid in a vacuum desiccator. Samples thus prepared were stored in tightly stoppered bottles.

<sup>9</sup> The writers are indebted to H. C. Diehl, of the Bureau of Plant Industry, stationed at Wenatchee, Wash., for procuring this material and forwarding it to Washington, D. C.

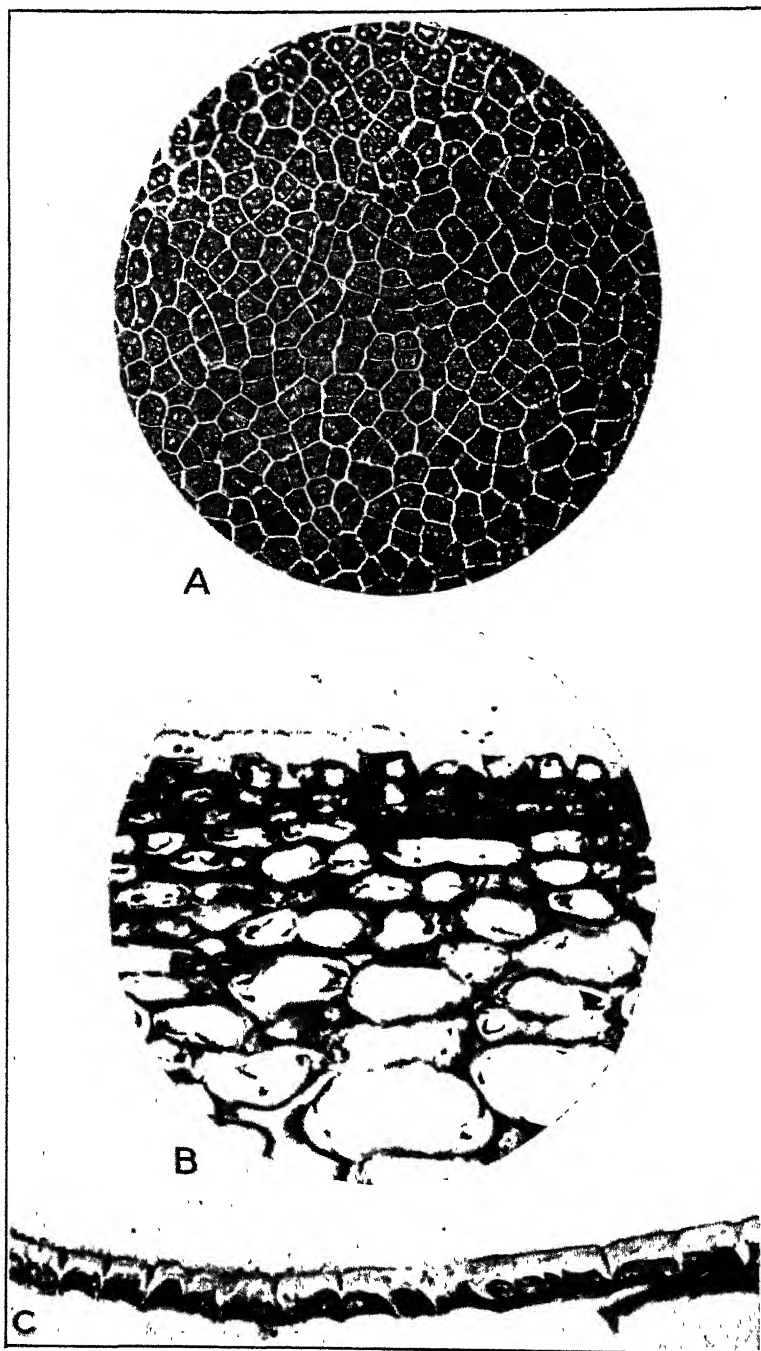


FIGURE 1.—Cuticular, epidermal, and subepidermal structure of Rome Beauty apple (New York).  
X 150: A, Surface view of cuticle, showing the underlying cellular structure of the epidermis; B,  
cuticle and section of the epidermis and adjacent tissues, showing thickness and extent of cuticle  
between epidermal cells; C, section of cuticle and all or a portion of the epidermal cells after  
removal with tweezers following treatment with dilute HCl. (These sections were kindly  
prepared by V. A. Pease, to whom the writers wish to express their thanks)

## DETERMINATION OF TOTAL ETHER EXTRACT

The samples of waxy cuticle obtained in the manner described were placed in fritted Jena-glass thimbles and dried for two hours at 103° to 105° C. The samples in the thimbles were then placed in Soxhlets and extracted continuously for 45 hours with pure anhydrous ether. The flasks were changed and the samples again extracted for 15 hours with double-distilled 95 per cent alcohol. The residue obtained by evaporating the alcohol was dried and extracted three times with ether at the boiling temperature. The ether solution was filtered through ashless filter paper into the flask containing the original ether extract. The combined ether extracts were evaporated to dryness and the residues dried one and one-half hours to constant weight in an air oven at 105°. The combined dried residue constitutes the total ether extract. This was found to consist principally of ursolic acid, triacontane, and heptacosanol, together with small quantities of unidentified oily substances and plastid pigments.

## DETERMINATION OF URSOLIC ACID

Inasmuch as ursolic acid is a hydroxy acid and forms a monosodium salt, it can be determined quantitatively by titration with alcoholic sodium hydroxide. The determination was carried out by adding approximately 50 ml. of 95 per cent alcohol and 1 ml. of 0.1 per cent phenolphthalein solution to the total ether extract and bringing the mixture to a boil so as to completely dissolve the residue. The hot solution was then titrated with approximately N/20 alcoholic sodium hydroxide, which was standardized at frequent intervals against benzoic acid. Blanks were run with each series of titrations and the correction applied to the final values.

## DETERMINATION OF OILY FRACTION

The oily fraction, consisting mainly of triacontane and heptacosanol, undoubtedly contained small quantities of true oily substances and plastid pigments; but, on account of the very small quantity of total ether extract, the separation and quantitative determination of the individual constituents were obviously impossible. The weight of the oily fraction was obtained by subtracting the weight of ursolic acid from the weight of the total ether extract.

## CALCULATION OF RESULTS

It was impracticable, for obvious reasons, to compute the analytical data on a basis of dry weight. The results are therefore reported on the basis of quantity of substance present on a unit area of apple surface, arbitrarily taken as 100,000 sq. mm. This area is equivalent to the area of 5 apples each 80 mm. in diameter, or to the area of 10 apples each 56.5 mm. in diameter. Since the apple surface is a curved one and the degree of curvature depends upon the size of the fruit, it was necessary to measure the equatorial diameter of each apple used in order to obtain the data necessary to calculate the total area of the experimental sample. For the calculation of the area of individual disks, it was necessary to apply the well-known geometrical formula for determining the area of the curved surface of a zone of one base. According to this formula, the area of the surface of a zone is equal to the product of its altitude ( $H$ ) and the circumference of a great circle ( $\pi D$ ), or

$$S = H \cdot \pi D.$$

In the case of the apple disk the altitude could not be measured directly but was calculated from the radius ( $R$ ) of the whole apple and from the radius ( $r$ ) of the disk removed therefrom. The final formula, therefore, is

$$S = (R - \sqrt{R^2 - r^2}) \cdot \pi D.$$

#### PROGRESSIVE CHANGES IN SURFACE CONSTITUENTS DURING GROWTH AND STORAGE

The data showing progressive changes in surface constituents of the fruit during the period of growth and while in storage at 32° F. are assembled in Table 1. From this table it may be seen that the quantities of ursolic acid, oily fraction, and total ether extract present at maturity and at the end of the storage period are invariably greater than those found in the early stage of growth for all varieties studied, for both the years 1926 and 1927. Data showing changes during storage are not available in all cases, but wherever given they indicate, with one exception, that the constituents increase from the time of picking to the end of the storage period. The progressive changes in constituents during growth and storage are brought out clearly in Figures 2 and 3.

While the quantities of both ursolic acid and the oily fraction become larger with increasing maturity, the oily fraction increases at a faster rate than does the ursolic acid; hence with advancing maturity there is in general a progressive increase in the percentage of the oily fraction in the total ether extract. (Table 1, columns 12 and 13.)

It is evident that most of the ursolic acid is deposited early in the growth of the apple, whereas the oily fraction, which is approximately 20 per cent lower than the ursolic acid in the early stage, is deposited at a uniformly higher rate throughout the later stages. This results in a gradual shift toward higher values of oily fractions (Table 1, columns 12 and 13) and lower values of ursolic acid in the total ether extract as the apple matures. A corresponding shift in the physical properties of the natural waxy coating must also accompany the shift in the ratio of the oily fraction to ursolic acid.

The change in physical properties which the waxy coating of apples undergoes as the fruit ripens may be a factor of some importance in the formation of certain odorous constituents. As the ratio of oily fraction to ursolic acid shifts, it is undoubtedly accompanied by a corresponding change in the physical properties of the natural waxy coating. Since the ursolic acid is dry, powdery, and porous and the oily fraction is pasty and greasy, it is natural to assume that the coating would become less permeable to gases as the percentage of oily fraction increases with advancing maturity.

A decrease in permeability might restrict the exchange of carbon dioxide and oxygen to such an extent that an intercellular-gas condition would be reached similar to that found by Thomas,<sup>10</sup> in which certain concentrations of these gases resulted in the formation of abnormal quantities of acetaldehyde.

<sup>10</sup> THOMAS, M. THE CONTROLLING INFLUENCE OF CARBON DIOXIDE. V. *Biochem. Jour.* 19: 947-947. 1925.

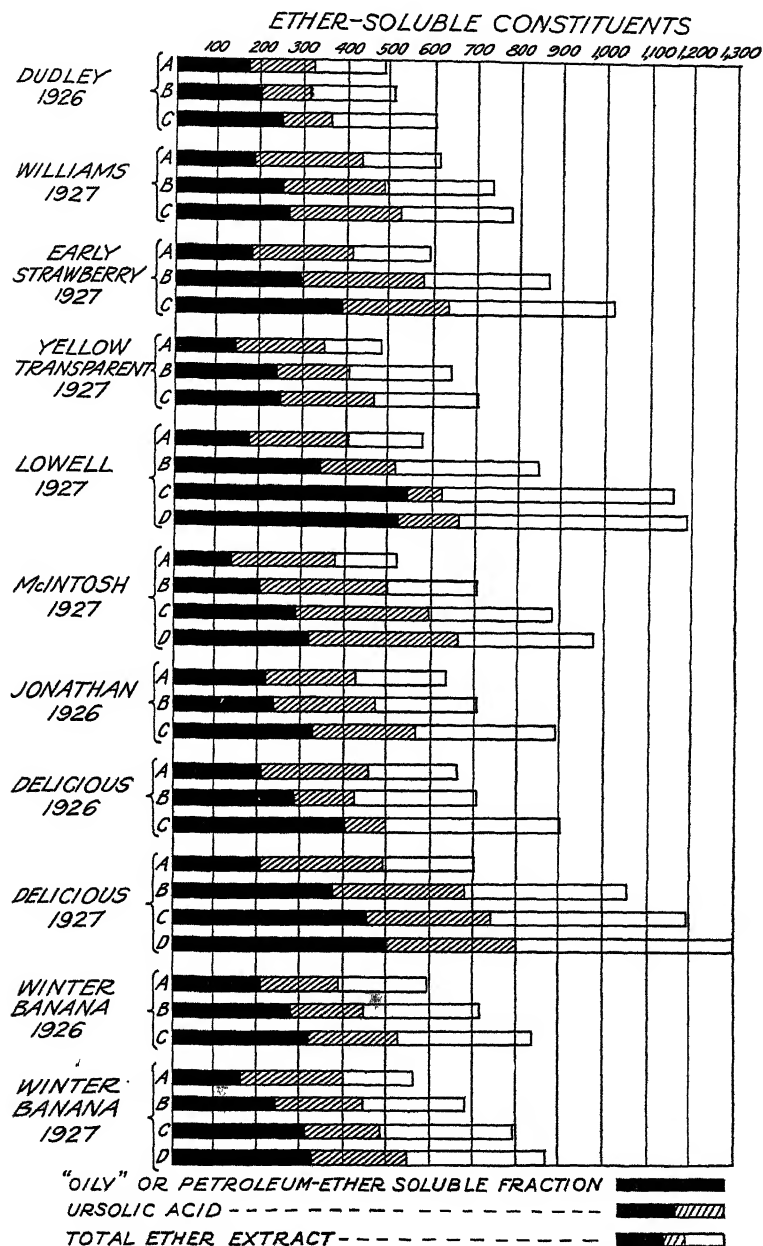


FIGURE 2.—Progressive changes in constituents during growth and storage expressed as milligrams per 100,000 sq. mm. of apple surface. A, B, C, and D refer to the early, medium, mature, and storage stages, respectively, shady side





FIGURE 3.—Progressive changes in constituents during growth and storage expressed as milligrams per 100,000 sq. mm. of apple surface. A, B, C, and D refer to the early, medium, mature, and storage stages, respectively, shady side

TABLE 1.—*Progressive changes in the ether-soluble surface constituents of apple varieties during maturation and in storage*

Variety, year, and stage of growth	Date of sampling	Age from blooming	Min- imum and maximum diameters of apples used	Total area of 75 disks	Constituents per 100,000 square millimeters of surface area						Only or petroleum- ether soluble frac- tion in total ether extract	
					Ursolic acid		Only or petroleum- ether soluble frac- tion		Total ether extract		Shady side	Sunny side
					Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side		
Summer varieties: <sup>a</sup>		Days	Mm.	Sq. mm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Per cent	Per cent
Dudley (1926)—												
Early.....	6/15/26	47	43-50	19, 213	318.5	274.3	167.6	133.7	486.1	458.0	34.5	40.1
Medium.....	7/27/26	89	63-77	27,924	310.5	273.9	198.7	196.2	506.2	470.1	39.0	41.7
Mature.....	8/11/26	104	62-81	27,867	359.9	366.0	244.3	257.6	604.2	623.6	40.4	41.3
Williams (1927)—												
Early.....	6/9/27	50	35-39	30,002	423.7	365.5	181.7	152.9	611.4	518.4	29.7	29.5
Mature.....	7/19/27	90	56-70	28,093	489.8	460.3	248.5	231.7	738.3	712.0	33.6	35.4
Storage.....	9/7/27	140	56-67	28,104	516.6	468.9	202.9	255.8	779.5	723.7	33.7	35.3
Early Strawberry (1927)—												
Early.....	6/6/27	47	31-35	31,135	407.9	335.0	178.6	152.6	586.5	487.6	30.5	31.3
Mature.....	7/18/27	89	47-56	28,526	577.1	432.3	291.7	240.3	868.8	681.6	33.6	36.6
Storage.....	9/14/27	147	47-56	28,508	630.8	467.5	386.8	341.0	1,017.6	808.5	38.0	42.2
Yellow Transparent (1927)—												
Early.....	6/6/27	47	30-39	30,733	345.7	330.4	140.6	139.0	480.3	400.4	28.9	29.6
Mature.....	7/11/27	82	54-67	28,185	408.0	323.9	234.9	234.9	642.0	612.9	36.5	36.5
Storage.....	9/13/27	146	51-65	28,234	459.7	459.7	243.7	243.7	703.4	703.4	34.6	34.6
Lowell (1927)—												
Early.....	6/6/27	47	30-36	31,044	404.2	361.4	171.1	194.5	575.3	555.9	29.8	35.0
Mature.....	7/21/27	92	55-65	28,175	508.9	308.9	338.6	338.6	847.5	847.5	38.9	38.9
Storage.....	8/23/27	125	62-90	27,881	619.8	619.8	538.4	538.4	1,158.2	1,158.2	46.5	46.5
Fall varieties: <sup>a</sup>												
Mcintosh (1927)—												
Early.....	6/9/27	50	33-41	29,999	383.3	375.0	133.0	128.9	516.3	501.9	25.8	25.3
Mature.....	7/21/27	92	51-64	28,252	594.5	480.8	202.4	200.3	706.9	681.1	28.4	28.4
Storage.....	9/7/27	140	60-78	27,950	598.8	548.9	284.1	237.7	889.9	846.2	32.3	32.3
Jonathon (1926)—												
Early.....	6/24/26	56	32-43	30,037	490.1	408.8	215.0	172.8	635.2	581.6	33.8	29.7
Mature.....	7/29/26	91	43-57	28,583	477.0	420.3	235.7	241.4	706.7	671.7	33.4	37.4
Storage.....	9/25/26	149	60-76	27,998	507.3	500.1	324.1	355.6	891.4	855.6	36.4	41.6

<sup>a</sup> Grown on the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C.<sup>b</sup> Sample from 50 instead of 75 disks.<sup>c</sup> Mixed sample; no difference discernible between shady and sunny sides of same fruit.

TABLE 1.—Progressive changes in the ether-soluble surface constituents of apple varieties during maturation and in storage—Continued

Variety, year, and stage of growth	Date of sampling	Age from blooming of apples used	Mini- mum and maximum diameters of apples used	Total area of 75 disks	Constituents per 100,000 square millimeters of surface area								Only or petroleum- ether soluble frac- tion in total ether extract	
					Ursolic acid		Only or petroleum- ether soluble frac- tion		Total ether extract					
					Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side
Fall varieties—Continued.														
Delicious—														
1926:														
Early	6/15/26	47	29-38	20,026	454.7	207.5	662.2	624.4	31.3	632.2	31.3			
Medium	7/27/26	89	45-55	28,782	420.7	423.5	708.4	717.4	40.7	708.4	40.7			
Mature	10/6/26	160	63-73	27,983	505.7	508.9	904.6	919.6	44.1	919.6	44.1			
1927:														
Early	6/13/27	54	31-39	30,028	496.3	452.5	208.6	704.9	29.6	663.1	29.6			
Medium	7/26/27	97	47-61	28,423	682.1	614.6	370.5	358.8	35.2	973.4	35.2			
Mature	9/21/27	154	59-73	27,997	747.6	732.3	448.7	496.1	37.5	1,228.4	37.5			
Storage	1/24/28	279	60-72	26,004	800.3	786.3	500.3	1,300.6	38.5	1,332.0	38.5			
Winter Banana—														
1928:														
Early	6/16/28	48	34-42	20,072	386.6	334.2	297.7	184.6	34.9	518.8	34.9			
Medium	8/3/28	96	54-63	28,237	443.8	401.3	274.3	277.5	38.2	678.8	38.2			
Mature	9/8/28	132	61-78	27,931	525.2	506.8	312.9	315.4	37.3	838.1	37.3			
1927:														
Early	6/13/27	54	32-40	30,293	390.1	344.0	161.4	151.8	28.8	495.8	28.8			
Medium	7/26/27	97	47-60	28,402	442.3	348.1	230.2	214.7	35.1	562.8	35.1			
Mature	9/21/27	141	56-70	27,953	487.5	406.0	308.0	280.4	38.7	693.4	38.7			
Storage	1/24/28	279	61-77	27,949	540.0	455.8	323.5	358.2	37.3	814.0	37.3			
Grimes Golden—														
1928:														
Early	6/28/28	60	33-40	30,205	391.2	365.4	275.6	264.3	41.3	666.8	41.3			
Medium	7/27/28	89	42-49	29,021	402.5	388.0	370.1	351.1	47.9	739.1	47.9			
Mature	10/13/28	167	58-74	27,995	572.6	606.2	610.1	644.7	51.6	1,250.9	51.6			
1927:														
Early	6/16/27	57	29-39	30,769	413.4	413.4	191.4	191.4	31.6	604.8	31.6			
Medium	7/26/27	97	47-57	28,460	478.7	478.7	314.3	314.3	39.6	793.0	39.6			
Mature	9/21/27	152	55-71	28,051	588.9	588.9	500.6	500.6	45.9	1,089.5	45.9			
Storage	1/3/28	258	60-72	27,998	608.3	608.3	501.9	501.9	45.2	1,110.2	45.2			
Late varieties:														
Baldwin (1927)—														
Early	6/9/27	50	33-43	29,991	386.4	333.4	190.4	164.0	33.0	497.4	33.0			
Medium	8/1/27	103	55-69	28,135	470.4	382.0	255.2	254.5	34.7	636.5	34.7			
Mature	9/20/27	163	65-83	27,936	579.4	499.8	322.6	301.7	35.8	821.5	35.8			
Storage	1/14/28	269	67-84	27,823	594.8	495.2	357.1	370.3	39.4	981.9	39.4			

Arkansas Black— 1926:	6/15/26	47	30-37	20,662	515.5	491.3	204.2	201.8	719.7	693.1	28.4	20.1
Early	8/7/26	100	49-60	28,442	626.6	569.6	316.4	317.8	943.0	887.4	33.6	35.8
Medium	9/25/26	149	61-73	27,980	722.3	634.0	395.6	482.1	1,117.9	1,116.2	35.1	43.2
Mature												
1927:	6/13/27	54	30-36	31,079	480.1	438.0	189.2	192.1	699.3	630.1	28.3	30.5
Early	7/25/27	96	47-57	28,928	510.9	578.0	270.0	305.3	848.0	816.2	37.4	37.4
Medium	9/26/27	159	60-75	27,970	694.5	600-6	397.2	390.1	1,021.7	996.7	38.9	39.7
Mature	2/8/28	294	60-72	28,007	713.6	674.7	459.5	515.5	1,173.1	1,100.2	39.2	43.3
Storage												
Rhode Island Greening (1927)—	6/9/27	50	36-44	29,653	364.1	344.6	163.9	167.9	528.0	512.5	31.0	32.8
Early	7/25/27	96	55-67	28,169	401.9	401.9	237.0	237.0	698.9	698.9	38.1	38.1
Medium	9/19/27	152	63-84	27,835	403.0	403.0	376.7	376.7	823.6	823.6	44.2	44.2
Mature	2/8/28	294	60-70	27,870	403.9	403.9	376.7	376.7	840.6	840.6	41.8	41.8
Storage												
Ben Davis (1926)—	6/24/26	56	33-42	30,238	430.0	366.4	209.0	243.4	629.0	609.8	33.2	39.9
Early	8/7/26	100	46-58	28,502	421.7	418.9	219.7	291.3	671.4	680.2	37.2	38.4
Medium	10/26/26	180	60-75	27,934	502.7		400.2		902.9		44.3	
Mature												
Paragon (1926)—	6/15/26	47	32-41	20,144	478.5	445.0	211.5	179.4	690.0	624.4	30.6	28.7
Early	8/3/26	96	50-60	28,332	528.7	522.7	310.6	300.1	830.3	823.1	37.0	36.5
Medium	10/28/26	182	63-79	27,877	627.0	433.7	433.7	409.7	1,000.7	823.1	40.9	
Mature	2/21/27	298	64-77	27,931	702.0	650.4	570.3	517.7	1,272.3	1,207.1	44.8	45.4
Storage												
Rome Beauty (1926)—	6/24/26	52	34-42	29,827	454.7	394.6	127.1	168.0	581.7	562.6	21.8	20.9
Early	8/4/26	93	48-60	28,470	456.8	434.0	264.0	185.7	720.8	614.7	36.6	30.0
Medium	11/2/26	183	66-81	27,842	552.8	518.0	382.2	407.7	934.6	925.6	40.9	44.0
Mature												
York Imperial— 1926:	6/28/26	60	37-50	29,353	369.0	380.6	223.5	217.4	592.5	596.6	37.7	36.2
Early	7/29/26	91	48-60	28,535	400.5	406.5	273.7	312.5	671.2	719.0	40.6	43.5
Medium	11/2/26	187	67-83	27,870	514.2	493.5	470.0	406.5	984.2	902.0	47.8	45.1
Mature												
1927:	6/16/27	57	31-35	31,148	389.7	362.7	193.5	184.3	583.2	517.0	33.2	33.7
Early	8/15/27	117	50-59	28,411	415.4	415.4		283.7	759.3	699.1	40.6	40.6
Medium	10/17/27	180	60-76	28,042	558.4	420.8	506.4	508.5	1,064.8	929.3	47.6	51.7
Mature	3/3/28	318	61-70	28,050	623.9	504.4	550.8	500.1	1,174.7	1,064.8	46.9	52.6
Storage												
Varities from Pacific Northwest:												
McIntosh • (1927)—	10/7/27		71-83	27,804	807.6	729.6	420.3	441.8	1,227.9	1,171.4	34.2	37.7
Mature												
Jonathan • (1927)—	10/7/27		64-76	27,908	542.8	530.9	299.0	298.9	842.4	829.8	35.6	36.0
Mature												
Delicious (1927)—	10/6/27		68-80	27,849	437.5	399.7	269.1	271.7	706.6	671.4	38.1	40.5
Mature												
Grimes Golden • (1927)—	10/6/27		62-71	27,974	658.2	610.1	421.0	444.0	1,079.2	1,054.1	39.0	42.1
Mature												
Winter Banana • (1927)—	9/27/27		71-82	27,796	619.1	632.7	443.5	469.0	1,062.6	1,101.7	41.7	42.6
Mature												

<sup>a</sup> From British Columbia.  
<sup>c</sup> From Wenatchee, Wash.

<sup>b</sup> Sample from 50 instead of 75 disks.  
<sup>c</sup> Mixed sample; no difference discernible between shady and sunny sides of some fruit.

TABLE 1.—Progressive changes in the ether-soluble surface constituents of apple varieties during maturation and in storage—Continued

Variety, year, and stage of growth	Date of sampling	Age from blooming	Mini- mum and maximum diameters of apples used	Total area of 75 disks	Constituents per 100,000 square millimeters of surface area								Only or petroleum- ether soluble frac- tion in total ether extract	
					Unsoluble acid		Only or petroleum- ether soluble frac- tion		Total ether extract					
					Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side
Summer varieties: <sup>a</sup>		Days	Mm.	Sq. mm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Per cent	Per cent		
Arkansas Black * (1927)— Mature	10/27/27	48	69-80	27,816	542.5	533.5	392.3	361.4	634.8	897.9	42.0	40.6		
Esopus Spitzenburg * (1927)— Mature	10/28/27	154	58-75	27,931	474.0	474.0	451.5	451.5	925.5	925.5	48.8	48.8		
Mean summer (1927):														
Early		48			396.9	348.1	108.0	150.8	564.9	507.8	29.7	31.4		
Mature		96			523.7	480.1	328.4	318.6	852.0	708.7	37.6	38.8		
Storage		154			567.0	514.2	352.8	339.6	919.8	853.8	37.6	39.0		
Mean fall (1926):														
Early		53			413.2	369.5	226.4	207.2	639.6	588.6	35.3	35.8		
Medium		91			434.5	408.3	292.2	293.5	726.7	701.8	40.0	41.7		
Mature		152			542.7	531.2	411.5	431.6	954.2	902.8	42.4	44.0		
Mean fall (1927):														
Early		54			423.0	396.2	173.6	170.2	596.6	569.4	29.0	29.8		
Medium		94			526.9	480.6	281.0	272.0	808.5	752.6	31.6	32.6		
Mature		147			605.2	569.0	385.4	395.9	990.6	964.9	38.6	40.8		
Storage		272			653.6	619.4	410.6	434.6	1,061.3	1,054.0	38.4	41.2		
Mean late (1926):														
Early		52			447.5	415.6	195.1	202.0	642.6	617.3	30.3	32.8		
Medium		96			486.9	470.3	282.9	275.5	746.9	734.5	37.0	36.8		
Mature		176			583.8	549.2	416.3	432.1	1,000.1	981.3	41.9	44.1		
Mean late (1927):														
Early		53			405.1	369.7	184.2	177.1	589.3	546.8	31.4	32.5		
Medium		103			486.4	427.6	257.4	272.6	747.7	700.2	34.9	36.0		
Mature		161			558.3	486.0	398.2	408.2	954.5	894.3	41.6	45.6		
Storage		294			599.0	534.0	443.5	457.2	1,042.6	991.8	42.6	46.0		
Mean Pacific Northwest (1927):														
Mature					553.1	558.6	385.3	391.6	968.4	950.2	39.9	41.2		

<sup>a</sup> Grown on the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C.<sup>e</sup> From Wenatchee, Wash.

## DIFFERENCES BETWEEN SHADY AND SUNNY SIDES OF SAME FRUIT

In general, the shady side of an apple as compared with the sunny side of the same apple possesses larger quantities of ursolic acid and total ether extract at all stages of growth and during the storage period. In view of the fact that the shady side contains a larger quantity of total ether extract, and since there seems to be no significant difference in oily fraction between the two sides, it follows that the percentage of oily fraction in the total ether extract should be lower on the shady side, as is actually the case.

The differences between the shady and sunny sides that have just been discussed are of particular interest in connection with the changes in quantity of constituents accompanying maturation. It has been pointed out that quantities of the various constituents increase with increasing maturity, but it may be noted that the actual values for ursolic acid and total ether extract are slightly smaller on the sunny side, which is generally considered the most mature portion of the apple. On the other hand, the percentage of oily fraction in the total ether extract is higher on the sunny side, thus conforming with the rule that the percentage of oily fraction increases with maturity.

It may be concluded, therefore, not only that the percentage of oily fraction is a criterion of maturity when apples of different ages are compared, but also that the higher percentages of this fraction on the sunny side of the fruit indicate a more mature condition of the sunny side as compared with the shady side of the same fruit.

## DIFFERENCES BETWEEN FRUIT COLLECTED IN 1926 AND 1927

With respect to the mean values for the quantities of the various constituents representing the fall and late groups, it is apparent that no marked differences exist between the two years 1926 and 1927.

However, when the values for certain individual varieties are considered, it is found that some were higher in 1926, others higher in 1927, and still others practically the same for both years. For example, in comparing data for the shady side only, it is noted that Arkansas Black at picking maturity was higher in ursolic acid (722.3 : 624.5 mgm.) and in total ether extract (1,117.9 : 1,021.7 mgm.) for 1926 than for 1927, and practically identical in the oily fraction for the two years (395.6 : 397.2 mgm.). York Imperial, on the other hand, was lower in ursolic acid (514.2 : 558.4 mgm.) and in the oily fraction (470.0 : 506.4 mgm.), and consequently in the total ether extract (984.2 : 1,064.8 mgm.), for 1926 than for 1927. Delicious was markedly lower in ursolic acid (505.7 : 747.6 mgm.), somewhat lower in oily fraction (398.8 : 448.7 mgm.), and considerably lower in total ether extract (904.6 : 1,196.3 mgm.) for 1926 than for 1927. Winter Banana shows higher ursolic acid (525.2 : 487.5 mgm.) and higher total ether extract (838.1 : 795.5 mgm.) in 1926 than in 1927, and about the same oily fraction (312.9 : 308.0 mgm.) for the two years. Grimes Golden shows practically the same quantity of ursolic acid (572.6 : 588.9 mgm.) for the two years, but a much higher value in oily fraction (610.1 : 500.6 mgm.) for 1926, which in turn is responsible for the higher value for the total ether extract (1,182.7 : 1,089.5 mgm.) in 1926 as compared with that in 1927.

## DIFFERENCES AMONG VARIETIES AT PICKING MATURITY

It is obvious from a consideration of the data presented in Table 1 that varietal differences are found with respect to quantities of constituents present at the different stages of maturity. Limiting the present discussion to the mature stage, and averaging the data for the shady and sunny sides of 1926 and 1927 fruit, the following varietal arrangement with respect to quantities in milligrams per unit area of ursolic acid is obtained:

Dudley, 363; Yellow Transparent, 408; Rhode Island Greening, 463; Williams, 475; Winter Banana, 482.1; York Imperial, 497.2; Ben Davis, 502.7; Early Strawberry, 504.7; Baldwin, 519.6; Jonathan, 533.7; Rome Beauty, 535.4; McIntosh, 572.8; Grimes Golden, 589.2; Lowell, 619.8; Delicious, 623.6; Paragon, 627; and Arkansas Black, 645.4.

When the quantities of oily fraction are similarly averaged, the arrangement of the varieties is as follows: Yellow Transparent, 234.9; Williams, 250.1; Dudley, 251; Early Strawberry, 270.5; McIntosh, 290.7; Winter Banana, 306.4; Jonathan, 339.8; Baldwin, 342.2; Rhode Island Greening, 366.5; Rome Beauty, 395; Ben Davis, 400.2; Arkansas Black, 417.8; Paragon, 433.7; Delicious, 438.6; York Imperial, 472.8; Lowell, 538.4; and Grimes Golden, 564.

With respect to the total ether extract, the arrangement is as follows: Dudley, 613.9; Yellow Transparent, 642.9; Williams, 725.2; Early Strawberry, 775.2; Winter Banana, 788.6; Rhode Island Greening, 829.6; Baldwin, 861.8; McIntosh, 863.6; Jonathan, 873.5; Ben Davis, 902.9; Rome Beauty, 930.1; York Imperial, 970.1; Paragon, 1,060.7; Delicious, 1,062.2; Arkansas Black, 1,063.1; Grimes Golden, 1,153.2; and Lowell, 1,158.2.

## QUANTITIES OF CONSTITUENTS EXPRESSED AS PERCENTAGES OF THE TOTAL CONSTITUENTS FOUND AT THE MATURE STAGE AND AFTER STORAGE

The data showing the quantities of the different fractions at various stages of growth, expressed as percentages of the total corresponding constituents at maturity and after storage are presented in Tables 2 and 3. From these tables it may be seen that a very large proportion of the several constituents is formed at an early stage in the development of the apple.

Considering the quantity of ursolic acid present at the mature stage as 100 per cent, it is interesting to note that approximately 75 per cent is deposited by the time the early stage is reached, or about 50 days from blooming, at which time the fruit has attained a size approximately 30 to 50 mm. in diameter. For the same period of growth the quantity of oily fraction laid down represents about 50 per cent of the total quantity present at the mature stage. From the data in Table 2 it is apparent that approximately 65 per cent of the total ether-soluble constituents are deposited within the first 50 days of growth.

TABLE 2.—Surface constituents of apple varieties at different stages of growth expressed as percentages of corresponding constituents found at picking maturity

Variety, year, and stage of growth	Age from blooming	Trisolic acid		Oily or petroleum-ether soluble fraction		Total ether extract	
		Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side
<b>Summer varieties:<sup>a</sup></b>							
Dudley (1926)—	Days	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Early.....	47	88.5	74.9	68.6	71.3	80.4	73.4
Medium.....	89	86.3	74.8	81.3	76.2	84.3	75.4
Mature.....	104	100.0	100.0	100.0	100.0	100.0	100.0
Williams (1927)—							
Early.....	50	87.7	79.4	73.1	60.7	82.8	72.8
Mature.....	90	100.0	100.0	100.0	100.0	100.0	100.0
Early Strawberry (1927)—							
Early.....	47	70.7	77.5	61.2	61.2	67.5	71.5
Mature.....	89	100.0	100.0	100.0	100.0	100.0	100.0
Yellow Transparent (1927)—							
Early.....	47	84.7	81.0	59.8	59.2	75.6	73.0
Mature.....	82	100.0	100.0	100.0	100.0	100.0	100.0
Lowell (1927)—							
Early.....	47	65.2	58.3	31.8	36.1	49.7	48.0
Medium.....	92	<sup>b</sup> 82.1	<sup>b</sup> 82.1	<sup>b</sup> 62.9	<sup>b</sup> 62.9	<sup>b</sup> 73.2	<sup>b</sup> 73.2
Mature.....	125	100.0	100.0	100.0	100.0	100.0	100.0
Mean (1927)—							
Early.....	48	77.1	74.0	56.5	54.3	68.6	66.3
Mature.....	96	100.0	100.0	100.0	100.0	100.0	100.0
<b>Fall varieties:<sup>a</sup></b>							
McIntosh (1927)—							
Early.....	50	64.2	68.3	46.8	42.7	58.6	59.3
Medium.....	92	84.5	87.6	71.2	67.4	80.2	80.5
Mature.....	140	100.0	100.0	100.0	100.0	100.0	100.0
Jonathan (1926)—							
Early.....	56	74.0	81.7	66.3	48.6	71.2	68.0
Medium.....	91	83.0	84.0	72.7	70.7	79.3	78.5
Mature.....	149	100.0	100.0	100.0	100.0	100.0	100.0
Delicious—							
1926:							
Early.....	47	89.9	—	52.0	—	73.2	67.9
Medium.....	89	83.2	83.2	72.4	71.6	78.4	78.0
Mature.....	160	100.0	100.0	100.0	100.0	100.0	100.0
1927:							
Early.....	54	66.4	61.8	46.5	42.4	58.9	54.0
Medium.....	97	91.2	83.9	82.6	72.3	88.0	79.2
Mature.....	154	100.0	100.0	100.0	100.0	100.0	100.0
Winter Banana—							
1926:							
Early.....	48	73.6	65.6	66.4	58.5	70.9	62.9
Medium.....	96	84.5	78.7	87.7	88.0	85.7	82.2
Mature.....	132	100.0	100.0	100.0	100.0	100.0	100.0
1927:							
Early.....	54	81.9	84.7	52.4	50.9	70.4	71.3
Medium.....	91	90.7	85.7	77.7	72.0	85.7	80.9
Mature.....	141	100.0	100.0	100.0	100.0	100.0	100.0
Grimes Golden—							
1926:							
Early.....	60	68.3	60.3	45.2	41.0	56.4	50.3
Medium.....	89	70.3	64.0	60.7	54.4	65.3	59.1
Mature.....	167	100.0	100.0	100.0	100.0	100.0	100.0
1927:							
Early.....	57	<sup>b</sup> 70.2	<sup>b</sup> 70.2	<sup>b</sup> 38.2	<sup>b</sup> 38.2	<sup>b</sup> 55.5	<sup>b</sup> 55.5
Medium.....	97	<sup>b</sup> 81.3	<sup>b</sup> 81.3	<sup>b</sup> 62.8	<sup>b</sup> 62.8	<sup>b</sup> 72.8	<sup>b</sup> 72.8
Mature.....	152	100.0	100.0	100.0	100.0	100.0	100.0
Mean—							
1926:							
Early.....	53	78.4	69.2	57.5	49.4	67.9	62.3
Medium.....	91	80.2	77.5	73.4	71.2	77.2	74.4
Mature.....	152	100.0	100.0	100.0	100.0	100.0	100.0
1927:							
Early.....	54	70.7	71.2	46.0	43.6	60.8	60.0
Medium.....	94	86.9	84.6	73.6	68.6	81.7	78.4
Mature.....	147	100.0	100.0	100.0	100.0	100.0	100.0

<sup>a</sup> Grown on the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C.<sup>b</sup> Mixed sample; no difference discernible between shady and sunny sides of same fruit.



TABLE 3.—*Surface constituents of apple varieties at different stages of growth expressed as percentages of corresponding constituents found at the end of the storage period—Continued*

Variety, year, and stage of growth	Age from blooming	Ursolic acid		Oily or petroleum-ether soluble fraction		Total ether extract	
		Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side
Late varieties—Continued.	Days	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
York Imperial (1927)—							
Early.....	57	62.5	71.9	35.1	32.9	49.6	51.4
Medium.....	117		82.4		50.6	64.6	65.6
Mature.....	180	89.5	83.4	91.9	90.7	90.6	87.3
Storage.....	318	100.0	100.0	100.0	100.0	100.0	100.0
Mean (1927)—							
Early.....	53	68.3	69.6	42.2	39.6	57.0	55.6
Medium.....	103	82.4	80.4	63.4	60.8	72.2	71.1
Mature.....	161	93.2	91.2	89.7	90.2	91.8	91.0
Storage.....	294	100.0	100.0	100.0	100.0	100.0	100.0

## SUMMARY AND CONCLUSIONS

With the particular object of determining the progressive changes that occur in the composition of the waxlike coating on the surface of the apple, analyses were made of summer, fall, and late varieties at several stages of growth and after storage at 32° F.

The results of these analyses, expressed in milligrams per 100,000 sq. mm. of apple surface, indicate that in general there is an increase in ursolic acid, oily fraction, and total ether extract throughout the growing period and during storage.

The oily fraction increases at a faster rate than ursolic acid, thus explaining the progressive increase in the percentage of oily fraction in the total ether extract with advancing maturity.

A change in the physical properties of the natural waxy coating undoubtedly accompanies the shift in the ratio of oily fraction to ursolic acid. Such a change probably not only affects the permeability of the coating to gases but also influences the effectiveness of spray applications and the ease of spray-residue removal.

In general, larger quantities of ursolic acid and total ether extract are deposited on the shady side than on the sunny side of the same apple, whereas there seems to be no appreciable difference in quantity of oily fraction. As a result, the percentage of oily fraction in the total ether extract is lower on the shady side.

Comparative data for varieties collected in the years 1926 and 1927 indicate that values for ursolic acid, oily fraction, and total ether extract may show variations from year to year, depending most probably on whether the season was favorable or adverse to the normal development of a particular variety.

It has been established that there are considerable variations among varieties with respect to quantities of ursolic acid, oily fraction, and total ether extract found at picking maturity.

Considering the average quantity of each substance deposited by the time the early stage is reached (approximately 40 to 60 days from blooming) as a percentage of the amount of the same substance present at maturity and at the end of the storage season, respectively, the following approximate values are found: For ursolic acid, 75 and 68 per cent; for oily fraction, 50 and 45 per cent; and for total ether extract, 65 and 58 per cent, respectively.

# AN ANALYSIS OF THE EFFECTS OF DIPLODIA INFECTION AND TREATMENT OF SEED CORN<sup>1</sup>

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## INTRODUCTION

The effects of various seed-borne seedling diseases caused by dry-rot organisms, and the effects of seed disinfection upon field stands and acre yields of corn have been rather extensively investigated by a number of State experiment stations and the United States Department of Agriculture. The literature has recently been so fully summarized by Koehler and Holbert (6)<sup>2</sup> that a review and enumeration of the many references would here seem unnecessary. It may be concluded from published reports that the prevalence and seriousness of these diseases vary regionally and seasonally with climate and cultural practice. In Nebraska they seem to offer no important crop-production problem with seed corn that is selected with the usual care.

When heavily infected viable seed has been selected and planted experimentally, however, the customary effects of curtailed stands have followed. Likewise, disinfection of such seed with commercial organic mercury compounds has resulted in improved field germination.

The most obvious result of planting viable infected seed is a stand shortage due to the death of many individuals caused by the rot organisms before emergence. The extent to which surviving infected plants might be depressed in their growth and productivity does not appear to have been clearly established. It has been the primary purpose of the investigation herein reported to determine this for the progeny from seed which was naturally infected with the most common of these seedling-blight organisms, *Diplodia zeae* (Schw.) Lév. The procedure has been that of statistical analysis.

Under field-planting conditions the diseased plants from such seed grow interspersed among healthy plants. It may be assumed that such a population will be less uniform in development than one with fewer diseased plants. As a concise expression of such effects, the means and their coefficients of variability have been calculated for various plant characters in the case of progenies from treated and untreated diseased and nearly disease-free seed. The characters considered were seedling weight, plant height at three stages of growth, days from planting to silking, shrinkage of ear corn, shelling percentage, and grain yield. In addition, the effects of the disease and of seed treatment on germination, seedling emergence, lodging, suckers, barren plants, 2-eared stalks, and smut in the resulting crop were determined. A record was also kept of the individual development of plants which gave initial evidence of being diseased as compared with adjacent healthy plants.

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<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 749.

Supplemental to the investigations enumerated above concerning the comparative behavior of the progenies from diseased and disease-free seed, a study was made of the effects of seed treatment on farm-selected or "planter-box" seed corn.

#### EXPERIMENTAL METHODS OF THE STATISTICAL STUDY

The seed used in this investigation was supplied by J. R. Holbert, of the United States Department of Agriculture. It was a double cross of uniform hybrid origin known as Hybrid No. 365. Two lots of seed differing in respect to infection with *Diplodia zeae* had been selected by means of the individual ear germinator test as described by Holbert et al. (2). Although one contained approximately 90 per cent infected kernels and the other less than 0.5 per cent, both had the appearance of good seed. Hereafter these will be referred to as diseased and healthy seed. Half of each lot was thoroughly treated with the mercuric disinfectant known as Semesan Jr. at the rate of 2 ounces per bushel. Thus the four lots of seed concerned consisted of untreated and treated diseased seed and untreated and treated healthy seed.

A laboratory germination test of the diseased and healthy seed was made by the rag-doll method. Field germination was determined for all four lots of seed by counting the number of seedlings which had emerged above the surface of the ground at intervals of 9, 12, 15, and 18 days after planting. Because the exact number of seeds planted was known, the counts of emerged seedlings were readily converted into percentage of field germination.

The tests were conducted in 1929 on the experiment station farm. Planting was done by hand on May 16 in surface-planted plots containing either four or five rows spaced 3.5 feet apart. The seeds were dropped every 14 inches in the row. All rows were 39.7 feet long and, when no skips occurred, contained 34 plants. The results were based on the interior rows of the plots only, and two plants at either end of each row were discarded. Ten systematic replications were provided for all plantings, in order that variability due to soil heterogeneity would be equally distributed. A full stand would thus provide populations of either 600 or 900 plants from each of the plantings for statistical study according to whether the plots contained four or five rows.

Based on the difference in laboratory germination and on previous experiences with similar seed, it seemed probable that unequal stands would result from the various lots of seed, if planted at a single uniform rate. Data taken from such stands would measure variability caused not only by *Diplodia* or seed treatment, but also by a variable competition within plots of differing population densities.

It seemed desirable so to adapt the plantings that the data obtained would show specifically the variability caused by (1) *Diplodia* or treatment alone and (2) the combined effects of *Diplodia* or seed treatment and the associated stand differences. Consequently plantings were made at two rates, a normal rate and a double rate. The plots planted at the double rate were later thinned; those planted at the normal rate were not. To make the desired comparisons, a total of 10 replicate series of 8 plots each was required. Plots 1 and 2 were respectively planted to untreated and treated diseased seed at the standard rate of one seed every 14 inches in the row, and the data are

based on all plants surviving at the time records were taken. Plots 3 and 4 were similarly planted to untreated and treated healthy seed at a double rate, and the seedlings were thinned when approximately 10 inches high so that the number and distribution of plants were identical with those in plots 1 and 2, respectively. In thinning, the seedlings were removed systematically according to position in order to maintain the normal proportion of inferior plants.

The comparisons between plots 1 and 3, showing the effect of *Diplodia* seed infection, and between 2 and 4, showing any remaining effect of the infection following seed treatment, may be made on identical but imperfect stand bases. A comparison between plots 1 and 2 shows the effect of treating *Diplodia*-infected seed where the progenies differ with respect to both disease and stand due to the treatment of seed in plot 2.

Plots 5, 6, 7, and 8 were duplicates, respectively, of 1, 2, 3, and 4 as to kind of seed planted, but differed in that all were planted at a double rate and thinned as closely as possible to uniform stands of one plant every 14 inches. The surplus plants, as in the previous case, were removed systematically according to their position in the hill in order to avoid changing the normal ratio of healthy and diseased seedlings. Further adjustment to perfect stand conditions was made by basing records on only those plants which were situated at least two spaces from skips within the row. Thus relatively uniform conditions were provided all plants except in so far as the effects of the vacant spaces both within the row and in adjacent rows were not entirely eliminated. These vacant space effects would be more pronounced in case of the diseased seed because of the greater number of skips.

After thinning, the interior rows to be used in obtaining data were assigned numbers and each plant was given an individual number. The position of each plant was so recorded that its development might be traced from the seedling stage to maturity and the determination of its individual yield.

The seedlings which were removed upon thinning the double-rate plots were considered representative of the respective populations from which they were drawn and were saved for determining individual moisture-free seedling weights. The weights were taken for the entire seedling less the roots.

Plant heights were determined at three stages of development. For the first two stages, five and nine weeks after planting, measurements were taken from the ground level to the tip of the longest leaf. When the plants were fully grown, the measurement was made to the lowest tassel branch.

The date of emergence of the first silks on each plant was determined by observing the plants daily. Any variability in time required to reach this stage could then be determined from these data.

Counts of bent and broken stalks, stalk and ear smut, barren plants, and suckers were made when the crop reached maturity.

The ears were harvested separately at the beginning of the normal husking season and assigned their individual plant numbers. Field weights were taken and the ears dried to a moisture-free basis in an oven. Weights were again taken for each ear, after which the ears were shelled and the weight of oven-dry shelled corn determined. Shrinkage and shelling percentages were then calculated.

## CALCULATION OF BIOMETRICAL CONSTANTS

The biometrical constants used in the analysis of the data were obtained by the use of the following standard formulas:

$$\text{Mean} = M = GA + c \text{ (algebraic)}$$

$$c = \frac{\Sigma(fd) \text{ (algebraic)}}{N},$$

where  $GA$  = guessed average,  $c$  = correction of the guessed average,  $\Sigma(fd)$  = summation of frequency  $\times$  deviation from the guessed average, and  $N$  = number of variates.

$$\text{Standard deviation} = S.D. = \sqrt{\frac{\Sigma(fd)^2}{N} - c^2}$$

$$\text{Coefficient of variability} = C.V. = \frac{S.D. \times 100}{M}$$

$$P.E. \text{ of mean} = \frac{\pm 0.6745 S.D.}{\sqrt{N}}$$

$$P.E. \text{ of } S.D. = \frac{\pm 0.6745 S.D.}{\sqrt{2N}}$$

$$P.E. \text{ of } C.V. \text{ for a } C.V. \text{ value of 10 or less} = \frac{\pm 0.6745 C.V.}{\sqrt{2N}}$$

$$P.E. \text{ of } C.V. \text{ for a } C.V. \text{ value greater than 10} \\ = \frac{\pm 0.6745 C.V.}{\sqrt{2N}} \left[ 1 + 2 \left( \frac{C.V.}{100} \right)^2 \right]^{\frac{1}{2}}$$

$P.E. \text{ of a difference} = \sqrt{a^2 + b^2}$ , where  $a$  and  $b$  represent the probable errors of the separate values compared.

In Tables 15 and 16 the deviation from the mean method described by Hayes and Garber (1) was used.

In the discussion of the results, a difference between two comparable constants must be at least 3.2 times its probable error to be considered as significant. This value of the ratio of the difference to its probable error corresponds to odds of approximately 30 to 1 that the difference is not due to error in the selection of the random sample. In appraising the results, it is well to remember that the value 3.2 is arbitrary.

## EFFECT OF DIPLODIA ON THE VIABILITY OF THE SEED

The results of the laboratory germination test of the seed used in planting the plots are reported in Table 1. In reading the test, the kernels were classified into four groups according to the apparent vigor of the sprout, as follows: (1) "Strong," which included all kernels producing vigorous sprouts from unrotted kernels even though the kernel or sprout showed the presence of mold; (2) "weak," which included kernels that were partly rotted or that had distinctly less vigorous sprouts; (3) "dead sprouts," which included all kernels

producing sprouts that later had rotted; and (4) "dead kernels," which included all kernels that failed to germinate.

The total germination for diseased seed and healthy seed was, respectively, 72 and 91 per cent. The classification of each 100 kernels which germinated from the two lots of seed is also shown in Table 1. Of the kernels which germinated, diseased and healthy seed produced, respectively, 67 and 75 per cent strong sprouts. Likewise, of the germinating seeds, diseased seed produced 2 per cent more weak sprouts and 6 per cent more dead sprouts than did healthy seed.

TABLE 1.—*Laboratory germination test of Diplodia-infected seed and nearly disease-free seed used in the field experiments*

Character of germination	Germination percentages based on—			
	All seed planted		Viable seed	
	Diseased seed	Healthy seed	Diseased seed	Healthy seed
	Per cent	Per cent	Per cent	Per cent
Strong.....	48	68	67	75
Weak.....	16	18	22	20
Dead sprouts.....	8	5	11	5
Dead kernels.....	28	9		
Total viable.....	72	91		

#### EFFECT OF SEED INFECTION AND TREATMENT ON FIELD GERMINATION

The results of the observations of field germination are similar to those obtained in the laboratory, and are reported in Table 2. The final field stands 18 days after planting were: Diseased 52.4, treated diseased 71.1, healthy 89.0, and treated healthy 88.9 per cent. When compared with the results of the laboratory germination test, it will be observed that the 52.4 per cent field stand from the untreated diseased seed corresponds quite closely to the 48 per cent which germinated vigorously in the laboratory, while the 71.1 per cent field stand from treated diseased seed corresponds to the total of 72 per cent which germinated in the laboratory. The percentage of field stand from healthy seed, either untreated or treated, does not differ more than 2 per cent from the total germination in the laboratory.

The percentage of field stand was also determined at three dates previous to the time when emergence was considered as complete. The data in columns 7, 8, and 9, Table 2, show that the seedlings which did emerge from either untreated or treated diseased seed were not retarded, nor is there any evidence to show that the treatment of nearly disease-free seed stimulated emergence.

TABLE 2.—*Effects of Diplodia seed infection and of seed treatment upon the field germination of corn, determined 9, 12, 15, and 18 days after planting*

Kind of seed and treatment	Seeds planted	Percentage of seedling emergence on—				Percentage of the final emergence on—			
		May 25	May 28	May 31	June 3	May 25	May 28	May 31	June 3
Diseased, untreated.....	2,380	10.3	52.0	52.5	52.4	19.7	99.2	100.0	100.0
Diseased, treated.....	2,380	11.2	69.6	70.8	71.1	18.8	97.9	99.6	100.0
Healthy, untreated.....	3,400	14.0	87.2	89.1	89.0	16.4	97.6	100.0	100.0
Healthy, treated.....	3,400	14.4	87.2	88.5	88.9	16.2	98.1	99.6	100.0

## EFFECT OF SEED INFECTION AND TREATMENT ON THE PERCENTAGE OF INFERIOR PLANTS

At the time of the first cultivation, five weeks after planting, the stands were examined for inferior plants. Plants which were spindling, stunted, off color, or otherwise lacking in vigor were considered as inferior. The results of this examination are shown in Table 3. The respective percentages of inferior plants from diseased seed, treated diseased seed, untreated healthy seed, and treated healthy seed were 11.9, 3.7, 2.7, and 2.5 per cent. While the treatment of diseased seed reduced the proportion of conspicuously inferior plants, there would seem to be no material advantage resulting from the treatment of healthy seed in reducing the percentage of such inferior plants. Of the inferior plants found in the seedling stage, 20 per cent disappeared before maturity.

TABLE 3.—*Effects of Diploдия seed infection and seed treatment upon the percentage of inferior corn plants, observed five weeks after planting, June 20, 1929*

Kind of seed and treatment	Total plants	Inferior plants	
	Number	Number	Per cent
Diseased, untreated.....	1, 248	149	11. 9
Diseased, treated.....	1, 692	62	3. 7
Healthy, untreated.....	3, 025	83	2. 7
Healthy, treated.....	3, 023	76	2. 5

## FINAL RESULTS FROM INFERIOR PLANTS

Of the inferior plants noted in the seedling stage, 81 were so situated at maturity that they could be compared with adjacent normal plants. The results are reported in Table 4. In the seedling stage, the inferior

TABLE 4.—*Comparison of seedling height, mature height, and plant yield of inferior plants and adjacent normal plants, and all plants from which data were secured*

Kind of plants compared	Plants harvested	Average—		
		Seedling height	Mature height	Yield of shelled corn per plant
	Number	Inches	Inches	Grams
Inferior plants.....	81	10. 0	58. 1	74. 7
Normal plants.....	81	19. 7	71. 4	158. 9
All plants.....	a 3, 417	20. 3	68. 6	129. 4

<sup>a</sup> This number was slightly greater for the seedling heights due to loss of some plants by the time of maturity.

plants averaged 10 inches in height as compared with 19.7 inches for the adjacent normal plants. At maturity, the inferior plants averaged 58.1 inches, while the adjacent normal plants averaged 71.4 inches. The inferior plants averaged 74.7 gm. of shelled corn per plant as compared with 158.9 gm. for the adjacent normal plants, and 129.4 gm. as the mean for the entire population.

These data show that although the inferior plants were only 51 per cent as tall in the seedling stage as the normal plants, they were 81

per cent as tall at maturity. The individual grain yield of the inferior plants averaged 42 per cent less, while that of the adjacent normal plants averaged 23 per cent more than the mean yield of all the plants from which data were secured.

### VARIABILITY OF PLANT CHARACTERS

In order to facilitate analysis of the comparisons under study, the description (p. 724) of the eight plantings upon which all data are based is shown in summary form in Table 5. For the sake of brevity,

TABLE 5.—*Kind of seed, treatment, planting rate, and kind of stand for the eight plantings on which all variability and other field data are based*

IMPERFECT STANDS					
Plot No.	Number of replications	Kind of seed	Treatment	Rate of planting	Kind of stand
1	10	Diseased	None	Single	Unadjusted.
2	10	do	Semesan Jr.	do	Do.
3	10	Healthy	None	Double	Adjusted identical to plot 1.
4	10	do	Semesan Jr.	do	Adjusted identical to plot 2.
NEARLY PERFECT STANDS					
5	10	Diseased	None	Double	Thinned to approximate perfect stand.
6	10	do	Semesan Jr.	do	Do.
7	10	Healthy	None	do	Do.
8	10	do	Semesan Jr.	do	Do.

the two types of stands typified by the two groups of plots, 1 to 4 and 5 to 8, will be referred to as imperfect and nearly perfect even though the latter is known to have been somewhat defective.

With the exception of Table 9, where the data for only four plots are given, Tables 6 to 13 show the mean results and their coefficients of variability for all eight plantings. The differences between certain of these means and coefficients are also given. The probable errors and the ratios D/PE are shown for these differences as accepted evidence of significance.

### VARIABILITY IN SEEDLING HEIGHT AT AGE OF 5 WEEKS

In the imperfect stands (Table 6) the mean plant heights, five weeks after planting, for the progenies of diseased, treated diseased, healthy, and treated healthy seed were  $17.85 \pm 0.16$ ,  $19.77 \pm 0.10$ ,  $20.63 \pm 0.12$ , and  $20.42 \pm 0.08$  inches, respectively, while the corresponding coefficients of variability were  $28.63 \pm 0.71$ ,  $17.35 \pm 0.35$ ,  $17.66 \pm 0.49$ , and  $14.66 \pm 0.29$  per cent. *Diplodia* infection served to reduce the mean plant height  $2.78 \pm 0.20$  inches and increased the variability  $10.97 \pm 0.86$  per cent. The treatment of diseased seed increased the mean plant height  $1.92 \pm 0.19$  inches and reduced the coefficient of variability  $11.28 \pm 0.79$  per cent. Treatment of healthy seed resulted in an insignificant decrease of  $0.21 \pm 0.14$  inch in plant height and a decrease of  $3.0 \pm 0.57$  per cent in the coefficient of variability.



TABLE 6.—*Effects of Diplodia seed infection and seed treatment upon the mean height and variability of corn plants, five weeks after planting, June 20, 1929\**

## MEAN HEIGHTS AND THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Plants measured	Plant height	
			Mean	Coefficient of variability
		Number	Inches	
1	Diseased, untreated.....	428	17.85±0.16	28.63±0.71
2	Diseased, treated.....	598	19.77±.10	17.35±.35
3	Healthy, untreated.....	428	20.63±.12	17.66±.49
4	Healthy, treated.....	598	20.42±.08	14.66±.29

## MEAN HEIGHTS AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

5	Diseased, untreated.....	246	18.37±0.21	26.05±0.82
6	Diseased, treated.....	447	21.41±.12	17.34±.42
7	Healthy, untreated.....	574	21.52±.10	16.27±.33
8	Healthy, treated.....	562	21.28±.09	14.30±.29

## DIFFERENCES BETWEEN MEAN PLANT HEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Plant height			
	Mean	D/PE	Coefficient of variability	D/PE
Healthy, untreated (plot No. 3) minus diseased, untreated (Plot No. 1).....	Inches 2.78±0.20	13.90	-10.97±0.86	12.76
Diseased, treated (plot No. 2) minus diseased, untreated (Plot No. 1).....	1.92±.19	10.10	-11.28±.79	14.28
Healthy, treated (plot No. 4) minus healthy, untreated (Plot No. 3).....	-21±.14	1.50	-3.00±.57	5.26

## DIFFERENCES BETWEEN MEAN PLANT HEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Healthy, untreated (plot No. 7) minus diseased, untreated (Plot No. 5).....	3.15±0.23	13.70	-9.78±0.88	11.11
Diseased, treated (plot No. 6) minus diseased, untreated (Plot No. 5).....	3.04±.24	12.67	-8.71±.92	9.47
Healthy, treated (plot No. 8) minus healthy, untreated (Plot No. 7).....	-24±.13	1.85	-1.97±.44	4.48
Healthy, untreated (plot No. 7) minus diseased, treated (Plot No. 6).....	.11±.16	.69	-1.07±.53	2.02

\* All plots were either 4 or 5 row plots 39 feet 8 inches long. The normal planting rate was one kernel every 14 inches in rows 42 inches apart. Plots 1 and 2 were planted at a normal rate. Plots 3 and 4 were planted at a double rate and thinned to the same number and distribution of plants, respectively, as grew in plots 1 and 2. In the case of plots 1 to 4, the data are based on all plants in the interior rows regardless of stand. In the case of plots 5 to 8, two seeds were dropped in the place of one, followed by thinning to one plant in a place. The variability studies were based on all plants in the interior rows which were removed two or more spaces from vacancies in the row.

For the plots containing a nearly perfect stand the mean heights of the progenies from diseased, treated diseased, healthy, and treated healthy seed were  $18.37 \pm 0.21$ ,  $21.41 \pm 0.12$ ,  $21.52 \pm 0.10$ , and  $21.28 \pm 0.09$  inches. The corresponding coefficients of variability were  $26.05 \pm 0.82$ ,  $17.34 \pm 0.42$ ,  $16.27 \pm 0.33$ , and  $14.30 \pm 0.29$  per cent. *Diplodia* infection resulted in a decrease of  $3.15 \pm 0.23$  inches in mean plant height and an increase of  $9.78 \pm 0.88$  per cent in coefficient of

variability. The treatment of diseased seed increased the mean plant height  $3.04 \pm 0.24$  inches and reduced the coefficient of variability  $8.71 \pm 0.92$  per cent. Treatment of healthy seed was accompanied by a reduction of  $0.24 \pm 0.13$  inch in plant height and  $1.97 \pm 0.44$  per cent in the coefficient of variability. Comparing the height of plants from treated diseased seed with that from untreated healthy seed, when grown under fairly comparable stand conditions at either this or later stages of development, no important difference was found.

#### VARIABILITY IN PLANT HEIGHT AT AGE OF 9 WEEKS

Under imperfect stand conditions (Table 7) the mean plant height at the age of 9 weeks for the progeny from diseased, treated diseased, healthy, and treated healthy seed were, respectively,  $59.11 \pm 0.32$ ,  $61.81 \pm 0.22$ ,  $61.32 \pm 0.24$ , and  $60.81 \pm 0.21$  inches. Corresponding coefficients of variability were  $16.48 \pm 0.39$ ,  $12.52 \pm 0.25$ ,  $11.59 \pm 0.27$ , and  $12.32 \pm 0.25$  per cent. As in the seedling stage, disease lowered the mean height and increased the variability. Likewise seed treatment increased the mean height and lowered the variability of the progeny from diseased seed. Treatment did not significantly affect the crop from healthy seed.

The effects of seed infection and seed treatment under nearly perfect stand conditions were almost identical with those just described.

TABLE 7.—*Effects of Diplodia seed infection and seed treatment upon the mean height and variability of corn plants nine weeks after planting, July 16, 1929\**

#### MEAN HEIGHTS AND COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Plants measured	Plant height	
			Mean	Coefficient of variability
		Number	Inches	
1	Diseased, untreated.....	425	$59.11 \pm 0.32$	$16.48 \pm 0.39$
2	Diseased, treated.....	568	$61.81 \pm .22$	$12.52 \pm .25$
3	Healthy, untreated.....	412	$61.32 \pm .24$	$11.59 \pm .27$
4	Healthy, treated.....	585	$60.81 \pm .21$	$12.32 \pm .25$

#### MEAN HEIGHTS AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

5	Diseased, untreated.....	222	$59.22 \pm 0.45$	$16.73 \pm 0.32$
6	Diseased, treated.....	419	$62.20 \pm .26$	$12.70 \pm .30$
7	Healthy, untreated.....	531	$63.51 \pm .21$	$11.05 \pm .23$
8	Healthy, treated.....	534	$63.38 \pm .22$	$11.69 \pm .25$

#### DIFFERENCES BETWEEN MEAN PLANT HEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Plant height			
	Mean	D/PE	Coefficient of variability	D/PE
Healthy, untreated (Plot No. 3) minus diseased, untreated (Plot No. 1).....	Inches $2.21 \pm 0.40$	5.53	$-4.89 \pm 0.47$	10.40
Diseased, treated (Plot No. 2) minus diseased, untreated (Plot No. 1).....	$2.70 \pm .39$	6.92	$-3.96 \pm .46$	8.61
Healthy, treated (Plot No. 4) minus healthy, untreated (Plot No. 3).....	$-1.51 \pm .32$	1.59	$0.73 \pm .37$	1.97

\* See footnote, Table 6, for details of planting.

TABLE 7.—*Effects of Diplodia seed infection and seed treatment upon the mean height and variability of corn plants nine weeks after planting, July 16, 1929—Continued*

DIFFERENCES BETWEEN MEAN PLANT HEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Kind of seed and treatment	Plant height			
	Mean	D/P E	Coefficient of variability	D/P E
Healthy, untreated (Plot No. 7) minus diseased, untreated (Plot No. 5).....	<i>Inches</i> 4.29±0.50	8.58	-5.68±0.39	14.56
Diseased, treated (Plot No. 6) minus diseased, untreated (Plot No. 5).....	2.98±.52	5.73	-4.03±.44	9.16
Healthy, treated (Plot No. 8) minus healthy, untreated (Plot No. 7).....	- .13±.30	.43	.64±.34	1.88
Healthy, untreated (Plot No. 7) minus diseased, treated (Plot No. 6).....	1.31±.33	3.97	-1.65±.38	4.34

## VARIABILITY IN PLANT HEIGHT AT MATURITY

At maturity, under imperfect stand conditions (Table 8) the mean plant heights to the lowest tassel branch for progenies of diseased, treated diseased, healthy, and treated healthy seed were  $67.57 \pm 0.31$ ,  $67.50 \pm 0.23$ ,  $67.92 \pm 0.27$ , and  $68.45 \pm 0.24$  inches, respectively. Corresponding coefficients of variability were  $13.35 \pm 0.29$ ,  $12.00 \pm 0.25$ ,  $11.76 \pm 0.28$ , and  $12.44 \pm 0.25$  per cent. Diplodia infection reduced the mean plant height  $0.35 \pm 0.41$  inch and increased the coefficient of variability  $1.59 \pm 0.40$  per cent. The treatment of diseased seed lowered the mean plant height only  $0.07 \pm 0.39$  inch and lowered the coefficient of variability  $1.35 \pm 0.38$  per cent. These data indicate no significant effect from Diplodia or from treatment of either diseased or healthy seed upon the mean height, and only very slight effects upon the variability.

TABLE 8.—*Effects of Diplodia seed infection and seed treatment upon the mean height and variability of corn plants at maturity\**

MEAN HEIGHTS AND THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Plants measured	Plant height	
			Mean	Coefficient of variability
		<i>Number</i>	<i>Inches</i>	
1	Diseased, untreated.....	397	67.57±0.31	13.35±0.29
2	Diseased, treated.....	560	67.50±.23	12.00±.25
3	Healthy, untreated.....	412	67.92±.27	11.76±.28
4	Healthy, treated.....	568	68.45±.24	12.44±.25

MEAN HEIGHTS AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

5	Diseased, untreated.....	210	67.93±0.42	13.26±0.44
6	Diseased, treated.....	383	69.15±.28	11.78±.29
7	Healthy, untreated.....	507	70.11±.26	12.50±.27
8	Healthy, treated.....	499	69.63±.25	12.01±.26

\* See footnote, Table 6, for details of planting.

TABLE 8.—*Effects of Diplodia seed infection and seed treatment upon the mean height and variability of corn plants at maturity—Continued*

DIFFERENCES BETWEEN MEAN MATURE PLANT HEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Plant height			
	Mean	D/PE	Coefficient of variability	D/PE
Healthy, untreated (plot No. 3) minus diseased, untreated (plot No. 1).....	<i>Inches</i> 0.35±0.41	0.85	-1.59±0.40	3.98
Diseased, treated (plot No. 2) minus diseased, untreated (plot No. 1).....	-0.07±.30	.18	-1.35±.38	3.55
Healthy, treated (plot No. 4) minus healthy, untreated (plot No. 3).....	.53±.36	1.47	-.68±.38	1.79

DIFFERENCES BETWEEN MEAN MATURE PLANT HEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5).....	2.18±0.49	4.45	-0.76±0.52	1.46
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5).....	1.22±.50	2.44	-1.48±.53	2.79
Healthy, treated (plot No. 8) minus healthy, untreated (plot No. 7).....	-.48±.36	1.33	-.49±.37	1.32
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6).....	.96±.38	2.53	.72±.40	1.80

In the presence of a more nearly perfect stand (plots 5 to 8) the mean heights of plants at maturity were  $67.93 \pm 0.42$ ,  $69.15 \pm 0.28$ ,  $70.11 \pm 0.26$ , and  $69.63 \pm 0.25$  inches, respectively, from the planting of diseased, treated diseased, healthy, and treated healthy seed. The corresponding coefficients of variability were  $13.26 \pm 0.44$ ,  $11.78 \pm 0.29$ ,  $12.50 \pm 0.27$ , and  $12.01 \pm 0.26$  per cent. Thus *Diplodia* lowered the mean height  $2.18 \pm 0.49$  inches but had no significant effect upon variability. Treatment increased the mean plant height for diseased seed  $1.22 \pm 0.50$  inches and lowered the coefficient of variability  $1.48 \pm 0.53$  per cent.

Taking all of these data into consideration, it appears that both disease and treatment have had an almost negligible effect upon the mature progeny of *diplodia*-infected seed. The rather pronounced differences in variability in the seedling stage have been largely outgrown. Treating healthy seed had no significant effects.

## VARIABILITY IN MOISTURE-FREE SEEDLING WEIGHT

The mean moisture-free weights of seedlings grown from diseased (Table 9), treated diseased, healthy, and treated healthy seed were  $1.72 \pm 0.047$ ,  $1.96 \pm 0.039$ ,  $1.94 \pm 0.028$ , and  $2.00 \pm 0.026$  gm., respectively. Corresponding coefficients of variability were  $52.73 \pm 2.39$ ,  $51.57 \pm 1.73$ ,  $42.46 \pm 1.17$ , and  $42.04 \pm 1.08$  per cent. *Diplodia* infection reduced the mean seedling weight  $0.22 \pm 0.055$  gm. and raised the variability  $10.27 \pm 2.66$  per cent. The treatment of diseased seed increased the mean seedling weight  $0.24 \pm 0.061$  gm. but did not materially affect the coefficient of variability. Treatment had no significant effect on the progeny from healthy seed.

TABLE 9.—*Effects of Diplodia seed infection and seed treatment upon the mean moisture-free seedling weight of corn and its coefficient of variability, when weighings were made four weeks after planting, June 17, 1929*

## MEAN WEIGHTS AND THEIR COEFFICIENTS OF VARIABILITY

Plot No.	Kind of seed and treatment *	Plants weighed	Seedling weight	
			Mean	Coefficient of variability
		<i>Number</i>	<i>Grams</i>	
5	Diseased, untreated.....	172	1.72±0.047	52.73±2.39
6	Diseased, treated.....	310	1.96±.039	51.57±1.73
7	Healthy, untreated.....	409	1.94±.028	42.46±1.17
8	Healthy, treated.....	470	2.00±.026	42.04±1.08

## DIFFERENCES BETWEEN MEAN WEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY

Kind of seed and treatment	Oven-dry seedling weights			
	Mean	D/PE	Coefficient of variability	D/PE
Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5).....	<i>Grams</i> 0.22±0.055	4.00	-10.27±2.66	3.86
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5).....	.24±.061	3.93	-1.16±2.95	.39
Healthy, treated (plot No. 8) minus Healthy, untreated (plot No. 7).....	.06±.038	1.58	-.42±1.59	.20
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6).....	-.02±.048	.42	-9.11±2.09	4.36

\* The seedlings used in this test were systematically removed, at the time of thinning, from plots 5 to 8 which had been planted at a double rate

## VARIABILITY IN NUMBER OF DAYS FROM PLANTING TO SILKING

The mean number of days from planting to silking and the coefficients of variability for each of the eight plantings are shown in Table 10. The maximum difference in length of time required to reach the fruiting period did not exceed one day, and there were no important differences in variability. In no case did either *Diplodia* seed infection or seed treatment influence the mean silking period more than 0.7 day nor did differences in the coefficients of variability exceed 1 per cent. Most of the differences showed very slight significance.

TABLE 10.—*Effects of Diplodia seed infection and seed treatment upon the mean number of days between planting and silking of corn and its coefficient of variability\**

## MEAN NUMBERS OF DAYS AND THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Plants observed	Period to silking	
			Mean	Coefficient of variability
		<i>Number</i>	<i>Days</i>	
1	Diseased, untreated.....	395	75.03±0.12	4.85±0.12
2	Diseased, treated.....	551	74.98±.10	4.69±.10
3	Healthy, untreated.....	406	74.59±.10	4.17±.10
4	Healthy, treated.....	558	75.29±.08	3.88±.08

\* See footnote, Table 6, for details of planting.

TABLE 10.—Effects of *Diplodia* seed infection and seed treatment upon the mean number of days between planting and silking of corn and its coefficient of variability—Continued

MEAN NUMBERS OF DAYS AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Plot No.	Kind of seed and treatment	Plants observed	Period to silking	
			Mean	Coefficient of variability
		<i>Number</i>	<i>Days</i>	
5	Diseased, untreated.....	213	75.89±0.18	5.19±0.17
6	Diseased, treated.....	391	75.58±.12	4.50±.11
7	Healthy, untreated.....	501	75.61±.11	4.82±.10
8	Healthy, treated.....	494	75.89±.10	4.44±.10

DIFFERENCES BETWEEN MEAN NUMBERS OF DAYS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Period to silking			
	Mean	D/PE	Coefficient of variability	D/PE
Healthy, untreated (plot No. 3) minus diseased, untreated (plot No. 1).....	<i>Days</i> -0.44±0.16	2.75	-0.68±0.16	4.25
Diseased, treated (plot No. 2) minus diseased, treated (plot No. 1).....	-.05±.16	.31	-.16±.16	1.00
Healthy, treated (plot No. 4) minus healthy, untreated (plot No. 3).....	.70±.13	5.38	-.29±.13	2.23

DIFFERENCES BETWEEN MEAN NUMBERS OF DAYS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5).....	-0.28±0.21	1.33	-0.37±0.20	1.85
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5).....	-.31±.22	1.41	-.69±.20	3.45
Healthy, treated (plot No. 8) minus healthy, untreated (plot No. 7).....	.28±.15	1.87	-.38±.14	2.71
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6).....	.03±.16	.19	.32±.15	2.13

## VARIABILITY IN THE SHRINKAGE OF EAR CORN

Shrinkage determinations are of interest as suggesting comparative maturity of the crop. In the first four plots which were subject to imperfect stands (Table 11) the mean ear-corn shrinkage percentages for diseased, treated diseased, healthy, and treated healthy seed were  $28.44 \pm 0.32$ ,  $26.64 \pm 0.25$ ,  $28.11 \pm 0.27$ , and  $26.81 \pm 0.25$  per cent, respectively. The corresponding coefficients of variability were  $32.34 \pm 0.86$ ,  $32.95 \pm 0.74$ ,  $28.71 \pm 0.73$ , and  $32.92 \pm 0.73$  per cent. *Diplodia* seed infection insignificantly increased the shrinkage  $0.33 \pm 0.42$  per cent, while the treatment of diseased seed lowered the shrinkage  $1.80 \pm 0.41$  per cent.

Under uniform stand conditions, the mean shrinkage percentages were  $26.88 \pm 0.50$ ,  $25.27 \pm 0.29$ ,  $26.27 \pm 0.32$ , and  $26.48 \pm 0.33$  per cent, respectively, for plots 5 to 8. Associated coefficients of variability were  $39.66 \pm 1.51$ ,  $33.12 \pm 0.90$ ,  $39.44 \pm 0.98$ , and  $39.65 \pm 1.00$  per cent. Seed infection insignificantly increased the shrinkage  $0.61 \pm 0.59$  per cent, while treatment of diseased seed lowered the shrinkage  $1.61 \pm 0.58$  per cent. No differences in variability were statistically significant except where the treated diseased seed was involved. The variability of this planting was so outstandingly low as to suggest an abnormal result.

TABLE 11.—*Effects of Diplodia seed infection and seed treatment upon the mean shrinkage percentage of ear corn and its coefficient of variability*<sup>a</sup>

MEAN SHRINKAGE PERCENTAGES AND THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Ears weighed	Shrinkage	
			Mean	Coefficient of variability
		<i>Number</i>	<i>Per cent</i>	
1	Diseased, untreated.....	592	28.44±0.32	32.34±0.86
2	Diseased, treated.....	547	26.64±.25	32.95±.74
3	Healthy, untreated.....	405	28.11±.27	28.71±.73
4	Healthy, treated.....	559	26.81±.25	32.92±.73

MEAN SHRINKAGE PERCENTAGES AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

5	Diseased, untreated.....	207	26.88±0.50	39.66±1.51
6	Diseased, treated.....	374	25.27±.29	33.12±.90
7	Healthy, untreated.....	482	26.27±.32	39.44±.98
8	Healthy, treated.....	474	26.46±.33	39.65±1.00

DIFFERENCES BETWEEN MEAN SHRINKAGE PERCENTAGES AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Shrinkage			
	Mean	D/PE	Coefficient of variability	D/PE
Healthy, untreated (plot No. 3) minus diseased, untreated (plot No. 1).....	<i>Per cent</i> -0.33±0.42	0.79	-3.63±1.13	3.21
Diseased, treated (plot No. 2) minus diseased, untreated (plot No. 1).....	-1.80±.41	4.39	-.61±1.13	.54
Healthy, treated (plot No. 4) minus healthy, untreated (plot No. 3).....	-1.30±.37	3.51	4.21±1.03	4.09

DIFFERENCES BETWEEN MEAN SHRINKAGE PERCENTAGES AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5).....	-0.61±0.59	1.03	-0.22±1.80	0.12
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5).....	-1.61±.58	2.78	-6.54±1.76	3.72
Healthy, treated (plot No. 8) minus healthy, untreated (plot No. 7).....	.21±.46	.46	.21±1.40	.15
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6).....	1.00±.43	2.33	6.32±1.33	4.75

<sup>a</sup> See footnote, Table 6, for details of planting.

## VARIABILITY IN THE SHELLING PERCENTAGE OF EAR CORN

The data in Table 12 indicate that neither *Diplodia* infection nor the treatment of either diseased or healthy seed had any significant effect on the mean percentage of shelled corn. The mean shelling percentages for imperfect stand conditions were, respectively,  $80.95 \pm 0.26$ ,  $80.59 \pm 0.20$ ,  $81.19 \pm 0.20$ , and  $81.38 \pm 0.17$  for diseased, treated diseased, healthy, and treated healthy seed. The corresponding coefficients of variability were  $8.58 \pm 0.19$ ,  $8.39 \pm 0.17$ ,  $7.24 \pm 0.17$ , and  $7.31 \pm 0.15$  per cent, respectively. The ears from diseased seed, either untreated or treated, were slightly more variable as to shelling percentage than those from healthy seed.

TABLE 12.—*Effects of Diplodia seed infection and seed treatment upon the mean shelling percentage of ear corn and its coefficient of variability*<sup>a</sup>

## MEAN SHELLING PERCENTAGES AND THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Ears weighed	Shelling per cent	
			Mean	Coefficient of variability
		<i>Number</i>		
1	Diseased, untreated.....	392	80.95±0.26	8.58±0.19
2	Diseased, treated.....	544	80.59±.20	8.39±.17
3	Healthy, untreated.....	404	81.19±.20	7.24±.17
4	Healthy, treated.....	558	81.38±.17	7.31±.15

## MEAN SHELLING PERCENTAGES AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

5	Diseased, untreated.....	234	80.47±0.33	8.75±0.29
6	Diseased, treated.....	374	80.91±.23	8.08±.20
7	Healthy, untreated.....	480	80.03±.26	10.36±.23
8	Healthy, treated.....	472	80.49±.24	9.71±.21

## DIFFERENCES BETWEEN MEAN SHELLING PERCENTAGES AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Shelling per cent			
	Mean	D/PE	Coefficient of variability	D/PE
Healthy, untreated (plot No. 3) minus diseased, untreated (plot No. 1).....	0.24±0.33	0.73	-1.34±0.25	5.36
Diseased, treated (plot No. 2) minus diseased, untreated (plot No. 1).....	-.36±.33	1.09	-.19±.25	.76
Healthy, treated (plot No. 4) minus healthy, untreated (plot No. 3).....	.19±.26	.73	-.07±.23	.30

## DIFFERENCES BETWEEN MEAN SHELLING PERCENTAGES AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5).....	-0.44±0.42	1.05	1.61±0.37	4.35
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5).....	.44±.40	1.10	-.67±.35	1.91
Healthy, treated (plot No. 8) minus healthy, untreated (plot No. 7).....	.46±.35	1.31	-.65±.31	2.10
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6).....	-.88±.35	2.51	2.28±.30	7.60

<sup>a</sup> See footnote, Table 6, for details of planting.

In the case of the plots which approximated a full stand of plants, the mean shelling percentages of ears grown from diseased, treated diseased, healthy, and treated healthy seed were  $80.47 \pm 0.33$ ,  $80.91 \pm 0.23$ ,  $80.03 \pm 0.26$ , and  $80.49 \pm 0.24$  per cent, respectively. As was true of the imperfect stands, these means do not differ significantly from each other. The coefficients of variability for the same plots were  $8.75 \pm 0.29$ ,  $8.08 \pm 0.20$ ,  $10.36 \pm 0.23$ , and  $9.71 \pm 0.21$  per cent,



respectively. The ears grown from either untreated or treated healthy seed were significantly less uniform in shelling percentage than ears grown from corresponding diseased seed. There seems to be no explanation why ears from the more uniform stands should be more variable in shelling percentage than those from the less uniform stands. When the corresponding coefficients of variability were averaged for both kinds of stand, they were found to be  $8.67 \pm 0.17$ ,  $8.24 \pm 0.13$ ,  $8.80 \pm 0.14$ , and  $8.51 \pm 0.13$  per cent, respectively, for ears produced from the diseased, treated diseased, healthy, and treated healthy seed. If it may be assumed that these averages are reliable, then there would appear to be no real difference in the variability of the shelling percentages from the various lots of seed.

#### VARIABILITY IN THE YIELD OF GRAIN PER PLANT

The mean oven-dry yields of shelled corn per plant are reported in Table 13. Plants grown under imperfect stand conditions from diseased, treated diseased, healthy, and treated healthy seed produced the respective mean grain yields of  $158.43 \pm 2.12$ ,  $134.43 \pm 1.35$ ,  $159.98 \pm 1.88$ , and  $136.55 \pm 1.40$  gm. These results show a 1 per cent lower mean yield per plant from heavily *Diplodia*-infected seed than was obtained from almost identically distributed plants grown from healthy seed. This difference is not significant since the probable error is nearly twice the difference. Treatment of the diseased seed resulted in an increased stand at maturity of 39 per cent and a decrease in mean yield per plant of 15 per cent. This decrease was no doubt due to the increased competition in the plots having the thicker stand.

TABLE 13.—*Effects of Diplodia seed infection and seed treatment upon the mean yield of shelled corn per plant and its coefficient of variability\**

#### MEAN YIELDS AND THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Plants	Shelled corn per plant	
			Mean	Coefficient of variability
		<i>Number</i>	<i>Grams</i>	
1	Diseased, untreated.....	391	$158.43 \pm 2.12$	$39.14 \pm 1.25$
2	Diseased, treated.....	544	$134.43 \pm 1.35$	$34.60 \pm .76$
3	Healthy, untreated.....	404	$159.98 \pm 1.88$	$35.08 \pm .93$
4	Healthy, treated.....	558	$136.55 \pm 1.40$	$35.86 \pm .81$

#### MEAN YIELDS AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

5	Diseased, untreated.....	204	$131.86 \pm 2.29$	$36.80 \pm 1.39$
6	Diseased, treated.....	374	$111.80 \pm 1.51$	$38.64 \pm 1.09$
7	Healthy, untreated.....	470	$105.50 \pm 1.37$	$41.68 \pm 1.07$
8	Healthy, treated.....	472	$101.31 \pm 1.23$	$38.86 \pm .97$

\* See footnote, Table 6, for details of planting.

TABLE 13.—*Effects of Diplodia seed infection and seed treatment upon the mean yield of shelled corn per plant and its coefficient of variability—Con.*

DIFFERENCES BETWEEN MEAN YIELDS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Shelled corn per plant			
	Mean	D/PE	Coefficient of variability	D/PE
Healthy, untreated (plot No. 3) minus diseased, untreated (plot No. 1).....	Grams 1.55±2.83	0.55	-4.06±1.56	2.60
Diseased, treated (plot No. 2) minus diseased, untreated (plot No. 1).....	-24.00±2.51	9.56	-4.54±1.46	3.11
Healthy, treated (plot No. 4) minus healthy, untreated (plot No. 3).....	-23.43±2.34	10.01	.78±1.23	.63

DIFFERENCES BETWEEN MEAN YIELDS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5).....	-26.36±2.67	9.87	4.88±1.75	2.79
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5).....	-20.06±2.74	7.32	1.84±1.77	1.04
Healthy, treated (plot No. 8) minus healthy, untreated (plot No. 7).....	-4.19±1.84	2.28	-2.82±1.44	1.96
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6).....	-6.30±2.04	3.09	3.04±1.53	1.99

In the case of the adjusted stands of 44.9 per cent from untreated healthy seed and 62.0 per cent from treated healthy seed, the 38 per cent greater stand resulted in a decreased mean plant yield of 15 per cent. These data indicate that under conditions of less than optimum stand, changes in the number of plants per acre are accompanied by somewhat smaller inverse changes in the yield of individual plants. Since this holds true in about equal measure for both diseased and healthy seed, it seems probable that increased yields per acre resulting from seed treatment are largely due to stand improvement.

#### MISCELLANEOUS FIELD DATA

Individual plant notes concerning various plant characters of agronomic interest were taken on all plants involved in these studies. These are summarized in Table 14. It is evident that neither seed infection nor treatment very materially influenced the percentage of bent, broken, barren, or 2-eared stalks. Variations in the number of plants having either moldy or smutty ears did not exceed 1 per cent. While the variation in number of smutty stalks was as great as for any of the plant characters observed, the life history of the smut disease will not permit ascribing this to the effects of either *Diplodia* or treatment. The treatment of both diseased and healthy seed was accompanied by a somewhat lower percentage of plants developing suckers. Taking all of these data into consideration, it is not apparent how any of these miscellaneous effects could have economic importance.

TABLE 14.—*Effects of Diplodia seed infection and seed treatment upon various plant characters of corn*

Kind of seed and treatment	Population	Number of plants per hundred—							
		Bent	Broken	Smutted in—		Having suckers	Barren	Bearing 2-eared stalks	Bearing moldy ears
				Ear	Stalk				
Diseased, untreated.....	607	4.9	1.5	0.4	8.4	15.2	2.6	4.4	1.0
Diseased, treated.....	943	7.0	2.4	.9	7.8	10.5	2.4	2.3	.7
Healthy, untreated.....	919	7.2	2.4	.8	12.0	20.7	3.7	3.3	.9
Healthy, treated.....	1,087	8.1	2.4	.5	10.4	14.9	2.4	2.7	.4

## EFFECT OF DISEASE AND TREATMENT OF SEED ON ACRE YIELDS

The yields of grain have been determined on an acre basis for the various lots of seed tested under both conditions of stand. The comparative results are reported in Tables 15 and 16. The yields are calculated for the interior rows of the 4-row or 5-row plots without corrections for missing plants, and are averages of 10 systematic replications. The probable errors are indicated for the mean yields and for the differences between yields in certain important comparisons.

Under the conditions of a standard planting rate, the diseased and treated diseased seed produced stands of 43.4 and 60.4 per cent, respectively, and the corresponding yields were  $34.0 \pm 0.59$  and  $39.5 \pm 0.68$  bushels.

Under conditions of comparable stands adjusted to be essentially identical with those above, stands of 44.9 and 62.0 per cent from healthy and treated healthy seed yielded  $35.0 \pm 0.61$  and  $41.2 \pm 0.71$  bushels. Thus diseased seed producing a stand of 43.4 per cent yielded  $34.0 \pm 0.59$  bushels, while healthy seed adjusted to a comparable stand of 44.9 per cent yielded  $35.0 \pm 0.61$  bushels. This difference of  $1.0 \pm 0.9$  bushel is only 1.11 times the probable error and is not to be regarded as significant.

TABLE 15.—*Effects of Diplodia seed infection and seed treatment upon the yield of shelled corn per acre*

[The yields reported are for all plants in plots 1 to 4 and are based on the entire area of the plots]

## MEAN YIELDS • PER ACRE

Plot No.	Kind of seed and treatment	Stand	Mean yield per acre
1	Diseased, untreated.....	<i>Per cent</i>	<i>Bushels</i>
2	Diseased, treated.....	43.4	$34.0 \pm 0.59$
3	Healthy, untreated.....	60.4	$39.5 \pm .68$
4	Healthy, treated.....	44.9	$35.0 \pm .61$
		62.0	$41.2 \pm .71$

## DIFFERENCE BETWEEN MEANS

Kind of seed and treatment	Mean	D/P/E
Healthy, untreated (plot No. 3) minus diseased, untreated (plot No. 1).....	<i>Bushels</i>	
Diseased, treated (plot No. 2) minus diseased, untreated (plot No. 1).....	$1.0 \pm 0.9$	1.11
Healthy, treated (plot No. 4) minus healthy, untreated (plot No. 3).....	$5.5 \pm .9$	6.11
	$6.2 \pm .9$	6.89

• Yields in bushels per acre are reported on a basis of 14 per cent moisture content.

TABLE 16.—*Effects of Diplodia seed infection and seed treatment upon the yield of shelled corn per acre*

[The yields reported are for all the plants in the double-rate thinned plots and are based on the entire area of the plots]

MEAN YIELDS * PER ACRE			
Plot No.	Kind of seed and treatment	Stand	Mean yield per acre
		<i>Per cent</i>	<i>Bushels</i>
5	Diseased, untreated.....	68.3	45.0±0.66
6	Diseased, treated.....	93.0	47.5±.69
7	Healthy, untreated.....	89.5	47.0±.69
8	Healthy, treated.....	89.8	45.6±.67
DIFFERENCE BETWEEN MEANS			
Kind of seed and treatment		Mean	D/P E
		<i>Bushels</i>	
Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5).....		2.0±.95	2.11
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5).....		2.5±.95	2.63
Healthy, treated (plot No. 8) minus healthy, untreated (plot No. 7).....		-1.4±.96	1.46
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6).....		-.5±.98	.51

\* Yields in bushels per acre are reported on a basis of 14 per cent moisture content.

The treatment of diseased seed gave an increased stand of 39 per cent and an increased yield per acre of 16 per cent, while the treatment of healthy seed adjusted to an increased stand of 38 per cent gave an increased yield of 18 per cent.

The 5.5 bushels per acre increase due to the treatment of diseased seed is 6.11 times the probable error and is therefore to be regarded as a significant difference. The difference of 6.2 bushels in the yield from untreated healthy and treated healthy seed is 6.89 times the probable error.

Since the treatment of diseased and healthy seed, adjusted to equal stands, was accompanied by almost equal yield increases, it is evident that the increase in case of the diseased seed is due to the greater number of plants rather than to a greater mean yield from the individual plants.

In case of the plots in which an effort was made to approach a perfect stand by thick planting and thinning, there were no significant differences between any of the yields. The diseased, treated diseased, healthy, and treated healthy seed produced respectively,  $45.0 \pm 0.66$ ,  $47.5 \pm 0.69$ ,  $47.0 \pm 0.69$ , and  $45.6 \pm 0.67$  bushels per acre. Corresponding field stands were 68.3, 93.0, 89.5, and 89.8 per cent at maturity. With a 36 per cent greater stand in the case of treated as compared with untreated diseased seed, there was only the rather small difference of  $2.5 \pm 0.95$  bushels in yield. Where the basic stand was thinner, as in the case of plots 1 and 2, an almost identical percentage increase in stand resulted in a much greater and more significant yield increase.

## EFFECT OF TREATING FARM-SELECTED SEED

The foregoing tests have been concerned only with comparisons of seed corn which had been specifically selected on the one hand because of heavy natural infection with *Diplodia zeae* and on the other hand for freedom from such disease organisms. Neither extreme represented such seed as farmers pick for planting by ordinary selection methods. While four years' results from experiment station tests with farm-selected seed (Kiesselbach (3)) have been published, the additional data secured in 1929 with such seed are of interest by way of substantiating the earlier conclusions. Ten lots of seed obtained from Nebraska corn growers and eight lots from growers in other States were planted comparably, both with and without treatment, in three replicated series of field plots. The plots contained three rows of 15 hills each, spaced 42 inches apart. Planting was done by hand at the rate of three seeds per hill. Treatment consisted of thorough dusting with Semesan Jr. The crop harvested was stored until uniformly air dry, when it was shelled and grain yields were calculated. Detailed observations concerning plant development were also recorded for all plots. These together with the yields are summarized in Table 17.

TABLE 17.—Effects of seed treatment upon the development and grain yield from farm-selected seed corn obtained from 18 growers in Nebraska and other States, 1929

## SEED FROM NEBRASKA GROWERS

Variety	Seed source		Seed treatment	Field stand in—		Date in tassel	Mature height	Percentage of plants—				Inferior plants in—		Moldy ears	Shrinkage of ear corn	Yield of shelled corn per acre
	Grower	State or locality		Spring	Fall			Barren	Lodged or broken	With suckers	Smutty	Spring	Fall			
				Per cent	Per cent	July	Inches					Per cent	Per cent	Per cent	Per cent	Bushels
Hogue	Experiment station.	Nebraska:	Treated	90	89	77	88	10	52	28	3	6	2	1	8	46.8
		Lincoln	None	89	88	78	89	12	48	41	9	8	2	0	6	48.4
		Utica	Treated	97	87	78	92	9	67	24	9	2	4	0	8	45.9
Do	Booth	Utica	None	95	92	79	91	4	70	19	10	4	4	2	9	48.1
		Blair	Treated	95	91	78	97	14	65	10	12	2	2	0	6	40.5
Reid	Aye	Blair	None	94	92	79	98	15	57	13	11	4	1	1	9	45.3
		Wesner	Treated	99	91	75	95	21	46	9	19	5	4	0	6	37.2
Do	Roggenback	Wesner	None	95	91	75	94	30	44	6	7	2	2	1	5	39.3
		Elk City	Treated	98	92	75	92	17	57	7	12	2	4	0	7	37.3
Do	Wahlgren	Elk City	None	98	91	75	92	21	45	12	12	2	4	1	5	30.0
		Papillion	Treated	95	87	31	92	19	59	12	13	4	4	1	9	36.9
Do	Gramlich	Papillion	None	90	87	31	90	20	58	10	12	6	3	1	10	35.5
		Lincoln	Treated	97	87	25	90	10	61	10	13	3	2	1	10	42.4
St. Charles	Ward	Lincoln	None	97	87	24	88	9	54	4	16	5	5	1	9	41.9
		Havelock	Treated	93	91	26	93	13	62	11	18	6	3	0	6	40.1
Gold Mine	Erskine	Havelock	None	87	86	25	96	10	55	18	10	7	4	0	6	40.4
		Beatrice	Treated	97	92	26	86	16	60	17	6	3	4	1	11	33.2
Kuhn	Sonderegger	Beatrice	None	96	91	26	89	19	62	17	10	3	4	1	11	45.2
		North Platte	Treated	89	89	23	83	11	43	8	8	11	4	3	4	37.8
White	Substation	North Platte	None	99	92	23	80	11	51	6	13	3	2	1	2	37.9
			Treated	96	90	26	91	14	57	14	11	4	3	1	8	40.8
Average			None	94	89	26	91	15	55	16	13	5	3	1	7	42.1

TABLE 17.—Effects of seed treatment upon the development and grain yield from farm-selected seed corn obtained from 18 growers in Nebraska and other States, 1929—Continued

## SEED FROM GROWERS IN OTHER STATES

Variety	Seed source		Seed treatment	Field stand in—		Date in tassel	Percentage of plants—				Inferior plants in—		Moldy ears	Shrinkage of ear corn	Yield of shelled corn per acre
	Grower	State or locality		Spring	Fall		Barren	Lodged or broken	With suckers	Smutty	Spring	Fall			
Krug	Behensee	Iowa	Treated	Per cent 98	Per cent 92	July 30	11	55	3	14	Per cent 3	3	Per cent 1	Per cent 4	Bushed 45.0
Do	Pfister	Illinois	None	94	87	30	17	63	8	10	4	2	0	8	43.3
			Treated	92	85	27	10	62	3	17	4	1	0	0	45.9
			None	93	87	26	13	51	8	16	5	4	0	6	44.7
Reid	Wilson	Iowa	Treated	93	92	26	13	58	6	13	3	2	0	7	41.2
			None	94	92	26	13	55	12	14	3	0	1	7	40.0
			Treated	94	92	24	13	56	7	11	3	3	2	5	47.8
Colby	do.	Kansas	None	89	87	24	13	56	7	11	3	3	0	5	42.5
			Treated	88	87	24	13	44	6	16	4	3	0	1	36.4
Reid	Carter	Illinois	None	85	79	26	15	40	6	15	5	3	1	2	36.7
			Treated	87	87	22	18	39	14	15	3	3	0	0	32.5
Ioleaming	Smith	Iowa	None	92	89	22	9	31	10	8	2	2	0	1	43.5
Hi-Bred	Newlin	do.	Treated	97	92	17	7	33	6	11	3	4	3	0	45.0
			None	94	88	27	9	36	5	11	7	6	0	7	26.1
			Treated	82	82	20	23	63	9	14	7	3	0	5	24.6
Murdock		Minnesota	None	78	78	20	16	49	2	14	5	3	0		
Average			Treated	91	88	26	14	51	7	14	4	3	1	4	39.8
			None	90	86	26	14	48	7	13	4	4	1	5	39.6
Grand average			Treated	94	89	26	14	54	11	12	4	3	1	6	40.4
			None	92	88	26	14	52	12	13	4	3	1	6	41.0

While there were some differences among the various samples with respect to their reaction to seed treatment, due perhaps in part to chance variation, there was no very material effect on an average for all of the samples. Identical results were obtained for time of tasseling and ripening, plant height, percentage of inferior plants four weeks after planting and also at maturity, percentage of barren plants, moldy ears, shrinkage of ear corn, and shelling percentage. Slight but unimportant differences occurred in the percentage of plants affected with tillers, smut, and lodging. The field count showed 2 per cent more plants from treated seed in the spring and 1 per cent more in the fall than were obtained from untreated seed. The yields of air-dry shelled grain per acre without correction for missing plants were 41 bushels for untreated seed and 40.4 bushels for treated seed. This is an average difference of 0.6 bushel in favor of no treatment. These data confirm the negative results obtained at this station in other years.

#### EFFECT OF TREATING VARIOUS GRADES OF SEED CORN DURING A PERIOD OF YEARS

Three years of seed-corn-treatment tests with *Diplodia*-infected and nearly disease-free seed corn have now been completed at the Nebraska experiment station. In addition, various representative collections of farm-selected or planter-box seed have been tested for their response to disinfection during a 5-year period. Since all of the results obtained prior to 1929 have been published (3, 4), a mere summary of field stands and acre yields will be included here. The treatments in any year have consisted of one or more of the standard commercial organic mercury seed disinfectants. The number of farmer's samples has varied from 6 to 30 annually. The selected diseased and healthy seed have been of the same Illinois variety or hybrid in any given year, and was procured for these tests from J. R. Holbert, of the United States Department of Agriculture. The test plots have consisted of three or four rows with three seeds dropped in hills  $3\frac{1}{2}$  feet apart. Yields were based on the interior rows.

TABLE 18.—*Summary of seed-corn-treatment tests with Diplodia-infected, selected healthy, and farm-selected seed corn during a period of years, 1925-1929* <sup>a</sup>

#### PERCENTAGE OF FIELD STAND OBTAINED

Year	Percentage stand obtained from seed that was—					
	Diplodia infected		Nearly disease free		Farm selected	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
1925.....					79	79
1926.....					73	73
1927.....	80	89	95	95	87	85
1928.....	60	78	93	91	96	95
1929.....	65	76	95	94	89	90
Average for three years.....	68	81	94	93	91	90
Average for five years.....					85	85

<sup>a</sup> The tests with diseased and healthy seed were strictly comparable since germ-inator selected seed grown at Bloomington, Ill., was procured from J. R. Holbert of the U. S. Department of Agriculture each year. The source and number of samples of Nebraska farm-selected seed varied from year to year. The number of farmers whose corn was represented were as follows: 1925, 30; 1926, 30; 1927, 17; 1928, 6; 1929, 10.



TABLE 18.—*Summary of seed-corn-treatment tests with Diplodia-infected, selected healthy, and farm-selected seed corn during a period of years, 1925-1929—Con.*

YIELD PER ACRE IN BUSHELS OF AIR-DRY SHELLED CORN						
1925.....					39.4	39.1
1926.....					7.2	7.2
1927.....	57.9	62.2	64.7	66.7	55.0	53.9
1928.....	27.4	28.1	25.6	24.8	27.5	27.5
1929.....	35.1	43.5	41.1	40.2	42.1	40.8
Average for three years.....	40.1	44.6	43.8	43.9	41.5	40.7
Average for five years.....					34.2	33.7

The results reported in Table 18 indicate that during three years untreated healthy seed surpassed the untreated diseased seed 38 per cent (fig. 1) in the number of plants produced and 3.7 bushels, or 9.2 per cent, in the yield of grain per acre.



FIGURE 1.—Comparative development of the progenies from healthy (right) and Diplodia-infected (left) seed corn. While the diseased seed resulted in a much thinner stand and somewhat more variable population, the inferiority was scarcely apparent through a mere inspection from the end of the plots because a large percentage of the plants were fully normal.

Seed treatment increased the stand of the diseased seed 19 per cent and the grain yield 4.5 bushels per acre. Neither the healthy seed nor the farm-selected seed responded favorably to seed disinfection.

#### DISCUSSION

The results with germinator-selected Diplodia-infected and healthy seed, farm-selected seed, and seed treatment reported herein for the year 1929 conform with those of similar tests in other years. Climatic conditions were fairly normal during the growing season of 1929, and it seems probable that crop responses to seed infection and seed treatment would average approximately the same in further tests. Based on the records of the United States Weather Bureau at Lincoln, the mean temperatures for May, June, July, and August, 1929, were 60.0°, 70.4°, 77.4°, and 76.2° F., respectively. These were departures of -1.7°, -1.0°, 0.9°, and 1.8° from the normal means of the respective months. Corresponding precipitation during these four months was 3.28, 1.86, 5.32, and 3.65 inches, respectively. These

represent departures of  $-0.80$ ,  $-2.46$ ,  $1.47$ , and  $-0.06$  inches from the normal.

In these tests the effects of seed-infection with *Diplodia* were determined through a comparison of plants from infected seed with plants from healthy seed that had been selected for relative freedom from dry-rot organisms. The two lots of seed differed distinctly with respect to germinative power. In laboratory tests only 72 per cent of the diseased seed proved viable, and in the field only 52 per cent emerged and produced seedlings. Ninety-one per cent of the healthy seed was found viable and 89 per cent of a field stand was obtained. Disinfection of the diseased seed increased the field stand by 36 per cent, while it did not affect the stand from healthy seed. These data show that while the healthy seed surpassed the diseased seed by 26 per cent in the number of seeds germinating in the laboratory, it surpassed by 70 per cent in the number of plants emerging under field conditions. The difference represents failure of viable seeds to produce plants because of the disease.

In these tests with *Diplodia*-infected seed, the disease resulted in the death of so many of the young sprouts before emergence as to cause a decided impairment of field stand when the seed was planted at the normal rate for this region.

If it were necessary, as has not been found to be the case in Nebraska, for a corn grower to plant seed so heavily infected with *Diplodia* as decidedly to curtail the stand, seed disinfection should be beneficial. Under such circumstances it would also probably be advantageous to plant the seed at a sufficiently heavy rate above normal to compensate for the reduction in field germination. Irregularity in the distribution of plants would be greater than in equal stands from healthy seed, but this has been shown by Kiesselbach, Anderson, and Lyness (5) to be unimportant as a yield factor. Exaggerated results of acre-yield depression are likely to follow from experimental tests planted at less than accepted standard rates. For example, as shown in the paper just cited, in a 12-year test of various planting rates, with farm-selected seed, a drop in stand from three plants to two plants per hill in checked corn resulted in a yield reduction of 7 per cent, whereas a drop from two plants to one plant per hill lowered the yield 19 per cent below that of two plants.

As indicated in these tests with farm-selected seed supplied by 18 growers, so-called planter-box seed responded to treatment with Semesan Jr. by a 2 per cent average increased field stand and one-half bushel lower acre yield. As a straight average for five years, farm-selected seed was unaffected in field stand and yielded one-half bushel less grain per acre when treated. At the North Platte substation, Zook and Jodon (?) obtained 1 bushel reduction in grain yield per acre on an average for seven organic mercury treatments applied to ordinary farm-selected seed. These are evidences that farm-selected seed is commonly not sufficiently infected with seedling-blight organisms to respond materially to treatment when planted at the prevailing standard rate under Nebraska conditions.

It appears evident from the statistical analysis of the plants from *Diplodia*-infected seed that the disease curtails the development of some of the surviving plants to such an extent as to reduce the mean plant height during growth approximately 2 or 3 inches and to increase somewhat the variability of the growing population. At

maturity, however, the relative differences have largely disappeared. Some of the affected plants may have been retarded early as a result of the disease but later outgrew the handicap. The tests supply further evidence that the disease is not systemic, as there is no greater percentage of diseased mature ears from infected than from healthy seed.

The statistical study of individual plants grown from diseased and healthy seed under conditions of essentially comparable stand suggest that the disease may have caused a doubtful reduction of 1 per cent in mean grain yield and a slight increase in the variability of individual production.

Under the conditions of these tests, stand reductions and resultant effects on yield are the only important consequences of seed infection with *Diplodia*. It is believed that in Nebraska the possible slight loss of viability due to this organism is negligible in its effects in the case of ordinary farm-selected seed planted at the customary rate per acre. The chief losses in viability are due to freezing injury, which is in no way related to infection with seed-borne disease organisms.

### CONCLUSIONS

The most pronounced effect of planting seed corn infected with the seedling-blight organism, *Diplodia zeae* (Schw.) Lév., is a reduction in field stand. Of the viable infected seed a high percentage may die as a result of the disease, when planted, before emergence above the soil surface. While the surviving plants are on an average slightly subnormal during their growth, they tend largely to overcome this handicap by maturity. Some of the plants which are most severely attacked by the disease during their early development remain decidedly inferior and less productive. The occurrence of inferior plants increases the variability of the entire population.

The reduced stands from *Diplodia*-infected seed result in a greater individual yield of grain per plant under Nebraska conditions due to lessened competition. For the same reason, the occurrence of inferior plants as a result of the seedling blight is partially compensated for by the increased production of neighboring plants. The degree to which compensation for such stand deficiencies may take place will depend considerably upon their extent and upon the climatic conditions of the season. The loss in grain yield per acre may ordinarily be expected to be in inverse though not proportional relation to planting rate up to the point of providing an optimum stand for the season. In these tests there was no significant difference between the acre yields from diseased and healthy seed when the stand from the latter was adjusted to correspond with the former in number and distribution of plants.

Treatment of viable *Diplodia*-infected seed with a suitable disinfectant may be expected to increase the resultant field stand materially. The variability of individual plant performance will thereby also be reduced. In case of deficient stand for maximum production such disinfection should also increase the yield of grain per acre. The mean grain yield per plant may actually be reduced because of more intensive competition due to closer spacing.

Seed corn selected by ordinary farm methods and secured directly from the growers has not responded significantly, on an average, to seed treatment when planted under field conditions.

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# THE ORDER, RATE, AND REGULARITY OF BLOOMING IN THE COTTON PLANT<sup>1</sup>

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## THE WORK OF OTHER INVESTIGATORS

Many studies have been made on the flowering of cotton. Most of these have been concerned with a certain number of plants or a given length of row (1, 7, 8, 15, 16, 17, 18).<sup>2</sup> Curves have been drawn showing fluctuations in the beginning of flowering and in the daily rate due to differences in varieties, culture, or fertilizer treatment. These have taken no cognizance of bud shedding, which might greatly affect the result, nor of the rate of blooming of individual plants, but only of totals, which, in the final analysis, have hinged mainly on vigor of growth. A few studies on individual plants have been reported (1, 4, 5, 10, 13).

Balls (5) depicted the order of blooming on an individual plant, showing that this order was in the form of a spiral. He took data on the growth of the main stem and found that the variations in growth were caused, in part at least, by the influence of night temperatures, and that the variations in the growth of the main stem were reflected in the variations in the number of flowers 28 to 29 days later. The correlation between growth and night temperatures (excluding periods when the maximum was above 36° C.) was  $+0.7843 \pm 0.0459$ . Since Balls notes the connection between early growth and blooming, some data on intervening phenomena—intervals between appearance of fruiting branches, appearance of squares, and the mean square period—are reviewed. The correlation between the intervals in all of these and in blooming is apparently high, though not perfect, as Balls would appear to indicate.

A slowing of the growth rate Balls attributes to "senescence" or to self poisoning, a phenomenon akin to "heat poisoning," which checks the growth during periods of extremely high temperature. He maintains that flower buds have a uniform rate of development but irregularities occur as a result of variations in night temperatures.

Hammond (9), however, found that the square period for buds appearing in May averaged 25 days, for those appearing in June 24 days, for those in July 24 days, and for those in August 25 days.

Loomis (11), in a study on length of squaring period in Acala and Pima cotton, found a variation from 32.4 to 34.5 days in 1924 and 32.08 to 35.06 in 1925 in Pima, and from 27.68 to 28.6 and 28.75 to 30.42 in Acala in the same years.

McClelland (12), working with plants of Cleveland Big Boll cotton growing in soil of constant moisture content, found a decided tendency toward regularity in blooming, the vertical intervals being approximately three days and the horizontal intervals approximately six days. This doubling of the interval indicated tendency for a bud at

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<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 763.



Ballard and Simpson (3) give some data on growth of cotton planted on different dates in Texas and South Carolina and show that the rate is speeded up by an increase in temperature.

Bailey and Trought (2), working with Sakel cotton in Egypt, found great regularity in blooming. If successive branches be lettered and successive nodes be numbered they show that [in theory]—

$$\left. \begin{array}{l} A^1 - A^2 = A^2 - A^3 = A^3 - A^4 \\ B^1 - B^2 = B^2 - B^3 = B^3 - B^4 \end{array} \right\} \text{and also that } \begin{array}{l} A^1 - B^1 = A^2 - B^2 = A^3 - B^3 \\ B^1 - C^1 = B^2 - C^2 = B^3 - C^3 \end{array} \text{ etc.}$$

This would be 100 per cent regular. Their results, however, showed some variation. They call attention to: (1) The remarkable constancy in blooming in individual plants; (2) the close approximation to the average for all plants; (3) the tendency to increased intervals on first nodes [vertical order] toward the top of the plant; (4) a like tendency to increased intervals between successive flowers [horizontal order] toward the end of each sympodium or fruiting branch; (5) the rhythmic nature of the flowering curves—high points occurring at intervals of 6.5 days; (6) the absence of correlation between intervals of blooming and vigor, length or thickness of sympodia, height of plant, or other characters or factors, including temperature; (7) the fact that bud shedding is a prime factor influencing flower curves made from blooms of a series of plants; and (8) that the period of square development is about 42 days rather than 28. (The American work on Egyptian cotton shows a square period of 30-35 days.)

They conclude that—

differences of intervals are fundamental, possibly genetic, and that the regular interval is undisturbed by rate or extent of elongation of the internode, by variations in temperature during time that differentiation of the bud primordia was taking place.

Their explanation of constancy is that all internodes contain equal numbers of cells, and that an increase in the interval of blooming in later stages of growth is due to an increase in the time required for the laying down of these cells. They offer no explanation, however, of daily variations.

Martin, Ballard, and Simpson (14) presented results of studies made on several varieties of cotton in 1921 and 1922 at Sacaton, Ariz., Greenville, Tex., and Charleston, S. C. Probably the intervals of appearance of successive fruiting branches bear a close relation to the "vertical order of blooming at first nodes" as found in the present paper—closer one would expect than to the vertical order at the fourth node or any outlying nodes.

Beckett (6) determined the length of the squaring period on different types of cotton and the intervals between horizontal and vertical squares. He found the mean square period for Indian, American, and Egyptian cotton to be three to four days shorter than Loomis had found for Acala. The figures for the mean square period for Egyptian cotton in all American studies vary from 30 to 35 days, and are intermediate between the 28 days of Balls and the 42 days of Bailey and Trought.

McNamara, Hubbard, and Beckett (13) summarize the blooming records of 25 Lone Star cotton plants. They recorded a horizontal interval of 6.2 and a vertical interval of 2.4 days. According to their



observations the mean square period varied from 25 to 28 days both in 1923 and in 1924. They claim that rapidity in blooming "is more closely associated with vigor of plants as affected by environmental conditions than with the advance in season."

#### EXPERIMENTAL MATERIAL AND METHODS

The work herein reported includes data from 24 plants grown at the main station, Fayetteville, Ark., in 1923, data from 240 plants grown at the same place in 1929, and data from 124 plants grown at the cotton branch station, Marianna, Ark., in 1929. The elevation of Fayetteville is about 1,450 feet and that of Marianna is about 200 feet; the latitude of Fayetteville is  $36^{\circ} 0' 14''$  and that of Marianna is  $34^{\circ} 0' 45''$  N. Several varieties were included in the experiments to get a fair representation of both short-staple and long-staple upland cotton. The cotton was spaced at 18 inches in 42-inch rows so as to give room for a fair degree of development and a goodly number of bolls per plant. However, at Fayetteville the drought was such that normal growth was not obtained or any considerable number of flowers per plant. The range of flowers per plant was from 4 to 30, with a mean of 14.9. At Marianna a better growth was obtained, the range of flowers per plant being 8 to 60, with a mean of 30.4.

#### EXPERIMENTS AT FAYETTEVILLE, 1923

Table 2 gives the results obtained at Fayetteville for eight plants each of three varieties in 1923. The distribution of the blooms and the means at different locations on the plants are included.

The horizontal intervals for Trice lie between 5.36 and 6.25; for Express, 5.33 and 6.20; and for Truitt, 5.82 and 6.20, showing the slower rate of blooming of the latter variety especially.

The range of intervals in the vertical order were for Trice, between 2.43 and 3.00; for Express, 1.45 and 2.47; and for Truitt, 2.00 and 2.52, the minimum for Express (1.45) being an inexplicably wide variation from all the other results obtained. To show better these varietal variations the data are rearranged in summary form in Table 3.

TABLE 2.—Frequency distribution of cotton bloom intervals in three varieties of cotton, Fayetteville, Ark., 1923

Item	Variety	Frequencies by intervals in days							Total	Mean interval and error
		3	4	5	6	7	8	9		
First and second node (A <sup>1</sup> -A <sup>2</sup> , B <sup>1</sup> -B <sup>2</sup> , etc.).	Trice.....		4	12	13	9	4		42	Days 5.93±0.101
	Express.....	1	4	15	11	6	3	1	41	5.73±.132
	Truitt.....		5	10	12	5	6	1	39	6.00±.143
Second and third node (A <sup>2</sup> -A <sup>3</sup> , B <sup>2</sup> -B <sup>3</sup> , etc.).	Trice.....		7	19	14	3	1		44	5.36±.092
	Express.....	1	5	10	12	5	3		36	5.67±.135
	Truitt.....	1	0	10	6	2	2	1	22	5.82±.187
Third and fourth node (A <sup>3</sup> -A <sup>4</sup> , B <sup>3</sup> -B <sup>4</sup> , etc.).	Trice.....		5	7	7	5	1		25	5.80±.153
	Express.....			6	9	1	3	1	20	6.20±.176
	Truitt.....			1	3	0	1		5	6.20±.312
Fourth and fifth node (A <sup>4</sup> -A <sup>5</sup> , B <sup>4</sup> -B <sup>5</sup> , etc.).	Trice.....			2	5	5			12	5.25±.135
	Express.....	1	0	0	1	1			3	5.73±1.200
	Truitt.....				1				1	6.00

TABLE 2.—*Frequency distribution of cotton bloom intervals in three varieties of cotton, Fayetteville, Ark., 1923—Continued*

Item	Variety	Frequencies by intervals in days							Total	Mean interval and error
		0	1	2	3	4	5	6		
At first node (A <sup>1</sup> -B <sup>1</sup> -C <sup>1</sup> -D <sup>1</sup> , etc.)	Trice		3	23	19	6			51	2.55±0.073
	Express		7	22	22	3	1		55	2.44±.077
	Truitt		4	26	11	4	1		46	2.39±.084
At second node (A <sup>2</sup> -B <sup>2</sup> -C <sup>2</sup> -D <sup>2</sup> , etc.)	Trice	1	7	17	16	5	1		47	2.43±.099
	Express		7	17	12	6	1		43	2.47±.102
	Truitt	2	7	12	13	6	0	2	42	2.52±.137
At third node (A <sup>3</sup> -B <sup>3</sup> -C <sup>3</sup> -D <sup>3</sup> , etc.)	Trice	1	4	12	12	3	1	1	34	2.56±.194
	Express	2	7	9	8	7	1		34	2.41±.146
	Truitt	2	2	2	2	3	0	1	12	2.50±.342
At fourth node (A <sup>4</sup> -B <sup>4</sup> -C <sup>4</sup> -D <sup>4</sup> , etc.)	Trice		6	7	5	4	2		24	2.54±.173
	Express	2	5	1	3				11	1.45±.219
	Truitt			1					1	2.00
At fifth node	Trice		1		1		1		3	3.00±.636
Average horizontal interval, all plants all branches.									290	5.79±.047
Average vertical interval, all plants all nodes.									403	2.48±.037

TABLE 3.—*Summary by varieties showing intervals of blooming in the cotton plant, Fayetteville, Ark., 1923*

Variety	Horizontal intervals				Vertical intervals		
	Location on plants	Plants	Counts	Mean interval and P. E.	Location on plants	Counts	Mean interval and P. E.
		Number	Number	Days		Number	Days
Trice	First to second nodes	8	42	5.93±0.101	First node	51	2.55±0.073
	Second to third nodes	8	44	5.36±.092	Second node	47	2.43±.099
	Third to fourth nodes	8	25	5.60±.153	Third node	34	2.56±.194
	Fourth to fifth nodes	8	12	6.25±.135	Fourth node	24	2.54±.173
Express	First to second nodes	8	41	5.73±.132	Fifth node	3	3.00±.636
	Second to third nodes	8	36	5.67±.135	First node	55	2.44±.077
	Third to fourth nodes	8	20	6.20±.176	Second node	43	2.47±.102
	Fourth to fifth nodes	8	3	5.00±.840	Third node	34	2.41±.146
Truitt	First to second nodes	8	39	6.00±.143	Fourth node	11	1.45±.219
	Second to third nodes	8	22	5.82±.187	First node	46	2.39±.084
	Third to fourth nodes	8	5	6.20±.312	Second node	42	2.52±.137
	Fourth to fifth nodes	8	1	6.00	Third node	12	2.50±.342
					Fourth node	1	2.00

## EXPERIMENTS AT FAYETTEVILLE, 1929

In 1929 counts were made on 240 plants of five varieties at Fayetteville. The season was very dry and growth was very unsatisfactory. Data were recorded between the dates July 28 and August 24, though blooming had practically ceased by August 16.

The number of blooms per plant ranged from 4 to 32, with a mean of approximately 15 per plant. As the plants were spaced at 18 inches in 42-inch rows, this average is quite low.

Table 4 gives a summary of the results obtained in both horizontal and vertical order with the several varieties, averages for the horizontal and vertical intervals for each variety, and for all varieties combined. Ratios between horizontal and vertical intervals are also shown. The longest intervals between blooms on fruiting branches were found on the Rowden and Express varieties. Triumph had a shorter interval than Trice, which has been reputed to be a rapid-

blooming variety. When the intervals in a vertical direction are considered, Delfos is found to be the most rapid bloomer and Triumph the slowest, the rate varying from 2.30 to 2.54 days, with an average for all varieties of 2.36 days. The intervals on different portions of the plants likewise varied widely (Table 5). For both years studied an increase in the interval is found toward the distal ends of the fruiting branches. In 1923 the increase was from 5.885 to 6.0, though the gain was not consistent. In 1929, when the number of plants was larger, a consistent increase from 5.898 to 6.226 was found.

TABLE 4.—Summary by varieties of data on rate and regularity of blooming in different varieties of the cotton plant, Fayetteville, Ark., 1929

Variety and number of plants	Horizontal intervals			Vertical intervals			Ratio of intervals X: 1
	Location on plants	Counts	Mean interval and P. E.	Location on plants	Counts	Mean interval and P. E.	
		Number	Days		Number	Days	
Rowden, 40 plants	First to second nodes..	133	6.06±0.063	First node..	185	2.26±0.058	2.68
	Second to third nodes..	55	6.40±.120	Second node..	105	2.54±.096	2.52
	Third to fourth nodes..	14	7.43±.223	Third node..	27	2.07±.222	3.59
	Fourth to fifth nodes..	163	5.52±.050	Fourth node..	213	2.35±.042	2.35
Delfos, 40 plants..	First to second nodes..	133	5.57±.061	First node..	175	2.22±.055	2.51
	Second to third nodes..	53	6.19±.097	Second node..	125	2.21±.084	2.80
	Third to fourth nodes..	9	6.33±.237	Third node..	29	2.83±.213	2.23
	Fourth to fifth nodes..	1		Fourth node..	4	2.75±.270	
Trice, 50 plants...	First to second nodes..	222	5.83±.057	First node..	289	2.48±.043	2.35
	Second to third nodes..	132	5.98±.069	Second node..	191	2.41±.058	2.48
	Third to fourth nodes..	50	5.98±.145	Third node..	94	2.17±.094	2.75
	Fourth to fifth nodes..	12	6.67±.380	Fourth node..	28	1.89±.208	3.53
Express, 50 plants	First to second nodes..	1	9.00	Fifth node..	5	1.00±.381	9.00
	Second to third nodes..	155	6.34±.060	First node..	238	2.46±.054	2.58
	Third to fourth nodes..	47	6.94±.098	Second node..	117	2.28±.083	3.04
	Fourth to fifth nodes..	3	6.33±.184	Third node..	20	1.18±.177	4.36
Triumph, 60 plants.	First to second nodes..	61	5.61±.140	Fourth node..	2	2.50±.239	
	Second to third nodes..	56	5.48±.143	First node..	100	2.61±.074	2.15
	Third to fourth nodes..	31	5.55±.199	Second node..	76	2.42±.087	1.31
	Fourth to fifth nodes..	10	5.60±.218	Third node..	62	2.39±.012	2.32
Average for:	Fifth to sixth nodes..	1	6.00	Fourth node..	12	3.00±.424	1.87
				Fifth node..	3	4.33±.662	
	Rowden.....	202	6.24		317	2.34	2.06
	Delfos.....	358	5.66		546	2.30	2.46
Total or average	Trice.....	417	5.93		607	2.37	2.50
	Express.....	205	6.48		377	2.34	2.77
	Triumph.....	149	5.55		253	2.54	2.18
		1,331	5.947		2,100	2.362	2.52

TABLE 5.—Summary by location on plants of results on regularity of blooming of cotton at Fayetteville, Ark., 1923 and 1929

Year and variety	Intervals of blooming between given nodes, horizontal order				Intervals of blooming between successive branches at respective nodes, vertical order				
	1-2	2-3	3-4	4-5	1	2	3	4	5
	Days	Days	Days	Days	Days	Days	Days	Days	Days
1923:									
Trice.....	5.93	5.36	5.60	6.25	2.55	2.43	2.56	2.54	3.00
Express.....	5.73	5.67	6.20	5.00	2.44	2.47	2.41	1.45	-----
Truitt.....	6.00	5.82	6.20	6.00	2.59	2.52	2.50	2.00	-----
Average.....	5.89	5.57	5.92	6.00	2.46	2.43	2.49	2.19	3.00
Total counts.....	122	102	50	16	152	132	80	36	3
1929:									
Rowden.....	6.06	6.40	7.43	-----	2.26	2.54	2.07	-----	-----
Delfos.....	5.52	5.57	6.19	6.33	2.35	2.22	2.21	2.83	2.75
Trice.....	5.83	5.98	5.98	6.67	2.48	2.41	2.17	1.89	1.00
Express.....	6.34	6.94	6.33	-----	2.46	2.28	1.18	2.50	-----
Triumph.....	5.61	5.48	5.55	5.60	2.61	2.42	2.39	3.00	4.83
Average.....	5.90	5.95	6.11	6.23	2.42	2.36	2.16	2.48	2.42
Total counts.....	724	423	151	31	1,025	664	328	69	12

In considering the vertical intervals at different distances from the main stems, less variation is noted than in the horizontal intervals, and some of these the writers are unable to explain. In 1923 the vertical interval at the fourth node out from the main stem was somewhat smaller than the others, and this was unduly increased (due to smallness of numbers mainly) at the fifth node; in 1929 the small interval was found at the third node. This increase in horizontal intervals agrees with the averages as calculated from Harland (Table 1), but there is disagreement in the holding constant of the vertical intervals. Harland's results showed consistent decrease, but perhaps this disagreement may be explained by the fact that he had a longer blooming period (129 to 137 days) and larger plants having 20 to 40 fruiting branches and as high as 15 blooms per fruiting branch and 175 (or more) potential blooms per plant.

#### THE EFFECT OF SEASON

Since data are available on two varieties, Trice and Express, covering two different seasons, the influence of season on intervals, if any, should be apparent. The monthly rainfall and the average maximum, minimum, and monthly temperatures for the summer months of 1923 and 1929 are given in Table 6.

TABLE 6.—*Rainfall and temperature records for June, July, and August, 1923 and 1929, in northwest Arkansas*

Item	June	July	August
1923—Rainfall, inches.....	5.20	3.53	2.43
Average maximum temperatures, °F.....	84.5	90.7	95.8
Average minimum temperatures, °F.....	65.0	67.3	70.1
Average monthly temperatures, °F.....	76.7	79.9	81.9
1929—Rainfall, inches.....	3.68	3.07	1.29
Average maximum temperatures, °F.....	82.7	94.4	95.9
Average minimum temperatures, °F.....	63.2	72.4	67.1
Average monthly temperatures, °F.....	76.2	81.2	80.8

\* For Eureka Springs; no data for Fayetteville.

A record of the blooming on Trice was begun on July 23 in 1923 and on July 28 in 1929. On Express the dates were July 26 and July 29, respectively. There were a few straggling blooms prior to these dates. Table 7 gives the mean interval measurements obtained. On both varieties there is a tendency toward a slight lengthening of the horizontal intervals and a shortening of the vertical intervals in 1929 as compared to the results obtained in 1923. More data will be needed, however, before a definite conclusion can be reached as to the effect of season on the intervals of blooming, though the increase in the horizontal intervals, especially with Express, is apparently significant.

TABLE 7.—Seasonal variation in the intervals of blooming of *Trice* and *Express* cotton plants, Fayetteville, Ark., 1923 and 1927

Variety and blooming measures	Location on plants	Counts and intervals in—				Gain or loss in intervals
		1923 (8 plants)		1929 (50 plants)		
		Counts	Mean interval and P. E.	Counts	Mean interval and P. E.	
Trice:		Number	Days	Number	Days	
Horizontal.....	First to second nodes.....	42	5.93±0.101	222	5.83±0.057	-0.10±0.118
	Second to third nodes.....	44	5.36±.052	132	5.98±.069	+.62±.115
	Third to fourth nodes.....	25	5.60±.153	50	5.98±.148	+.38±.214
	Fourth to fifth nodes.....	12	6.25±.135	12	6.67±.380	+.42±.403
Vertical.....	First node.....	51	2.55±.073	289	2.48±.043	-.07±.085
	Second node.....	47	2.43±.099	191	2.41±.058	-.02±.115
	Third node.....	34	2.56±.194	94	2.17±.094	-.39±.215
	Fourth node.....	24	2.54±.173	28	1.89±.208	-.65±.270
	Fifth node.....	3	3.00±.636	5	1.00±.381	-2.00±.741
Express:						
Horizontal.....	First to second nodes.....	41	5.73±.132	155	6.34±.060	+.61±.145
	Second to third nodes.....	36	5.67±.135	47	6.94±.098	+1.27±.167
	Third to fourth nodes.....	20	6.20±.176	3	6.33±.184	+.13±.254
	Fourth to fifth nodes.....	3	5.00±.840			
Vertical.....	First node.....	55	2.44±.077	238	2.46±.054	+.02±.094
	Second node.....	43	2.47±.102	117	2.28±.083	-.10±.131
	Third node.....	34	2.41±.146	20	1.18±.177	-1.23±.229
	Fourth node.....	11	1.45±.219	2	2.50±.239	+1.05±.324
Average, Trice:						
Horizontal.....		123	5.690±.064	416	5.92±.042	+.230±.076
Vertical.....		159	2.524±.053	607	2.37±.033	-.154±.062
Average, Express:						
Horizontal.....		100	5.780±.088	205	6.477±.052	+.697±.102
Vertical.....		143	2.366±.056	377	2.336±.044	-.030±.071

## PLANT NO. 14

AUGUST



## PLANT NO. 24

AUGUST



## DATES AND NUMBER OF BLOOMS

FIGURE 2.—Frequency of blooms in August of two cotton plants showing cyclic tendencies

## THE RHYTHMIC CYCLE OF COTTON BLOOMING

Bailey and Trought (2) called attention to the fact that flowering curves are rhythmic, high points occurring at intervals of 6.5 days. The explanation of the regular appearance of peaks in the curves lies in the regularity of blooming in the individual plants. In theory, if the vertical interval is 3 and the horizontal interval 6, and nothing disturbs the regularity, blooms should occur on any one plant only at 3-day intervals, and there would be no true daily curve for individual plants. If the regularity is upset (from whatever cause) and if the intervals are not exactly three or six days, nor yet in an exact ratio of 1:2, there are many blooms on intermediate days and the charted record becomes a curve, with peaks at intervals. Bud shedding also tends to interrupt the expected result. The fact that different plants begin blooming on different dates is, however, the main reason for a smoothing of these curves. (Fig. 2.)

If a majority of the plants began blooming on the same day, the tendency toward regularity would be great enough to account for peaks in the curves at regular intervals, though this does not explain why the high points occur near the 6-day interval rather than on the three.

A study of the flowering data from certain plants will illustrate the idea. Figure 2 gives the number of flowers occurring on the different days during August on the two plants used for illustration in the article by McClelland (12) to which reference has been made. The rhythmic period, as shown in Figure 2, varies from five to seven days, but probably averages very close to the mean length of the horizontal intervals as found at any given place or season on any given variety of cotton. A slight tendency toward a 3-day cycle is noted only in plant No. 24. On smaller plants, such as were used in 1929, a study

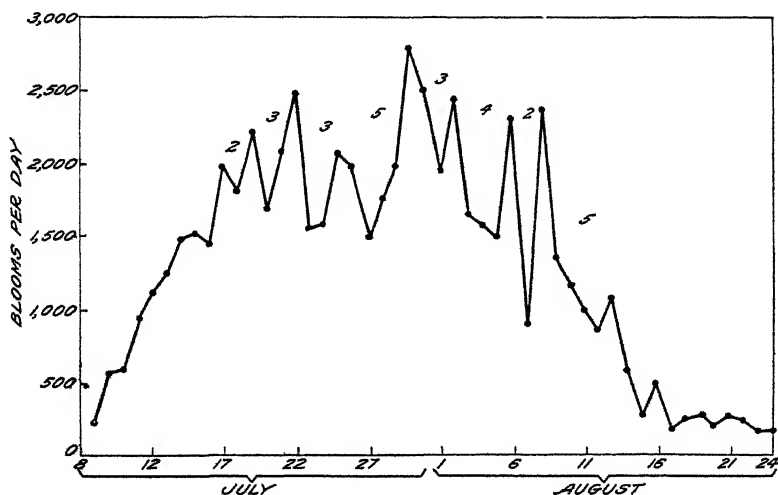


FIGURE 3.—Flowering curves of 30 check rows of Rowden cotton, Scott, Ark., July 8 to August 24, 1929

of this variation in blooming can hardly be made. The tendency toward a smoothing of the flowering curve with any larger number of plants is readily explained. If conditions are uniform and such that most of the plants begin blooming on the same day, and if they preserve the same degree of regularity, as it seems they should, then peaks in the curves at more or less regular intervals are bound to occur. Inspection of the data from 30 rows, 44 inches by 132 feet, of Rowden cotton in 1929 (fig. 3) reveals a great irregularity in the number of blooms per day but no cyclic tendency. With the 240 plants used at Fayetteville in 1929 the peaks occurred at an average of four days with one 3-day and one 5-day interval alternating with those of four days. (Fig. 4.)

#### RELATION OF VIGOR OF PLANT TO LENGTH OF BLOOMING INTERVAL

It was thought that the very unfavorable conditions at Fayetteville in 1929 might have influenced the intervals of blooming and that this influence would be most apparent in the plants of least vigor; in other words, that the intervals would be smaller in plants having

the larger number of flowers. To determine this point the average horizontal and vertical intervals for each of the 240 plants were computed. These were then grouped in classes with a class interval of 0.2. The range horizontally was from 4.7 to 7.5 days and vertically from 1.3 to 3.7 days, the few extreme variations being included in the outer groups.

The coefficients of correlation were: Between number of flowers per plant and average horizontal interval per plant,  $r = -0.132 \pm 0.0425$ ; between number of flowers per plant and average vertical interval per plant,  $r = -0.251 \pm 0.0415$ .

These correlations appear to be significant though very small, indicating that vigor of growth as shown by number of flowers per

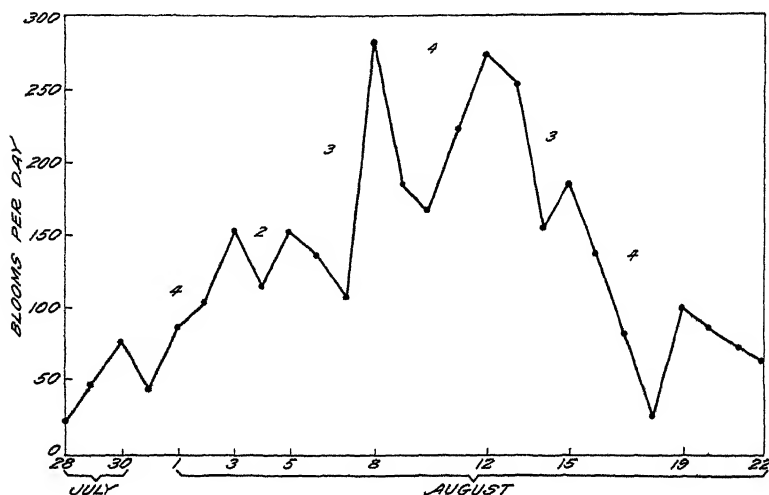


FIGURE 4.—Flowering curve of 240 cotton plants grown at Fayetteville, Ark., 1929

plant exercised a small influence in reducing the length of the blooming intervals.

#### EXPERIMENTS AT MARIANNA, 1929

In cooperation with the cotton branch station at Marianna records were kept on 124 plants in 1929. Plantings of Acala, Trice, and Express were made on May 6 and May 21, and one planting of Delfos was made on May 6. The Trice, Delfos, and Express varieties are common to the two tests of 1929.

Table 8 summarizes the results of the findings at the cotton branch station. With all varieties, the intervals increased horizontally to the interval between the third and fourth nodes, after which irregularity occurred. In the vertical order, it was generally found that there was little difference in intervals at first or second nodes, there was usually some shortening of intervals at the third nodes, and beyond this the results were more variable.

TABLE 8.—Summary by varieties showing rate and regularity of blooming in cotton plants, cotton branch station, Marianna, Ark., 1929

Variety, number of plants, and planting date	Horizontal intervals			Vertical intervals			Ratio of intervals X:1
	Location on plants	Counts	Mean interval and P. E.	Location on plants	Counts	Mean interval and P. E.	
		Number	Days		Number	Days	
Acala, 19 plants, May 6.	First to second nodes...	141	6.18±0.079	First node...	156	2.75±0.075	2.24
	Second to third nodes...	111	6.44±.085	Second node...	119	2.65±.087	2.43
	Third to fourth nodes...	63	6.75±.129	Third node...	86	2.50±.118	2.70
	Fourth to fifth nodes...	22	6.91±.208	Fourth node...	38	2.29±.211	3.01
	Fifth to sixth nodes...	8	6.50±.337	Fifth node...	9	2.78±.459	2.33
Acala, 18 plants, May 21.	First to second nodes...	117	5.95±.074	First node...	138	2.39±.061	2.49
	Second to third nodes...	78	6.26±.099	Second node...	88	2.42±.103	2.58
	Third to fourth nodes...	33	6.85±.141	Third node...	50	2.28±.177	3.00
	Fourth to fifth nodes...	5	6.00±.060	Fourth node...	15	1.80±.231	3.33
Trice, 20 plants, May 6.				Fifth node...	2	1.50±.239	
	First to second nodes...	189	5.62±.066	First node...	195	2.47±.056	2.27
	Second to third nodes...	149	5.68±.078	Second node...	157	2.43±.074	2.33
	Third to fourth nodes...	93	5.86±.110	Third node...	106	1.95±.102	3.00
	Fourth to fifth nodes...	45	5.82±.151	Fourth node...	64	2.55±.161	2.28
	Fifth to sixth nodes...	21	6.00±.187	Fifth node...	20	2.70±.262	2.22
Trice, 20 plants, May 21.	Sixth to seventh nodes...	3	5.00±.318	Sixth node...	6	1.83±.604	2.73
	First to second nodes...	130	5.53±.077	First node...	152	2.28±.060	2.42
	Second to third nodes...	76	5.86±.104	Second node...	87	2.33±.094	2.51
	Third to fourth nodes...	32	6.34±.180	Third node...	46	2.19±.163	2.89
	Fourth to fifth nodes...	12	5.83±.236	Fourth node...	18	2.45±.236	2.38
Express, 19 plants, May 6.	Fifth to sixth nodes...	4	5.50±.377	Fifth node...	9	1.14±1.140	4.82
	First to second nodes...	148	5.93±.077	First node...	164	2.49±.059	2.32
	Second to third nodes...	86	6.48±.104	Second node...	123	2.54±.087	2.55
	Third to fourth nodes...	42	6.67±.163	Third node...	65	2.35±.135	2.84
	Fourth to fifth nodes...	12	6.25±.357	Fourth node...	29	2.69±.237	2.32
Express, 9 plants, May 21.	Fifth to sixth nodes...	2	3.50±.238	Fifth node...	6	1.60±.451	2.19
	First to second nodes...	33	5.82±.162	First node...	53	2.57±.125	2.26
	Second to third nodes...	14	6.00±.361	Second node...	18	2.89±.175	2.07
Delfos, 19 plants, May 6.	Third to fourth nodes...	6	6.50±.545	Third node...	7	2.71±.589	2.40
				Fourth node...	3	1.67±1.051	
	First to second nodes...	164	5.24±.075	First node...	175	2.54±.059	2.06
	Second to third nodes...	126	5.50±.086	Second node...	131	2.38±.088	2.31
	Third to fourth nodes...	80	5.73±.107	Third node...	101	2.56±.107	2.23
Average:	Fourth to fifth nodes...	45	5.53±.157	Fourth node...	51	2.59±.174	2.13
	Fifth to sixth nodes...	9	6.33±.106	Fifth node...	24	2.00±.242	3.16
Acala, early		345	6.42		408	2.63	2.44
Acala, late		233	6.18		293	2.34	2.64
Trice, early		500	5.71		548	2.37	2.41
Trice, late		254	5.74		312	2.25	2.53
Express, early		290	6.20		387	2.48	2.50
Express, late		53	5.94		81	2.62	2.27
Delfos, early		424	5.46		482	2.48	2.20
Total or average		2,099	5.90		2,511	2.44	2.41

Trice is shown to be a faster bloomer than either Acala or Express. Delfos also had shorter horizontal intervals, but was only average in the length of the vertical intervals.

It is evident that there was reduction in horizontal intervals caused by later planting; in vertical intervals, two of the three varieties showed this reduction. In other words, the rate of blooming was accelerated by delaying the planting until the arrival of longer days and warmer weather.

#### THE EFFECT OF ALTITUDE ON BLOOMING INTERVAL

By comparing the data taken at Fayetteville with those from Marianna the effect of different environmental conditions may be observed. The comparison shown in Table 9 indicates a lengthening of the blooming intervals, horizontally, due to greater altitude and lower temperatures. On the other hand, the vertical intervals are shortened in Delfos and Express, but are slightly lengthened in Trice at the higher altitude.



TABLE 9.—*Effect of altitude on blooming intervals of three varieties of cotton*

Variety	Average horizontal intervals at—		Average vertical intervals at—	
	Fayetteville (elevation 1,450 feet)	Marianna (elevation 200 feet)	Fayetteville (elevation 1,450 feet)	Marianna (elevation 200 feet)
	<i>Days</i>	<i>Days</i>	<i>Days</i>	<i>Days</i>
Trice.....	5.93	<sup>a</sup> 5.72	2.37	<sup>a</sup> 2.32
Delfos.....	5.66	5.46	2.30	2.48
Express.....	6.48	<sup>a</sup> 6.16	2.34	<sup>a</sup> 2.50

<sup>a</sup> Average of the 2 plantings.

## SUMMARY AND DISCUSSION

Cotton exhibits a greater or less tendency toward regularity in its blooming. The regularity is interrupted by many different causes and the rate is influenced by variety, seasonal conditions, location, cultural methods, and possibly other factors.

The intervals between the appearance of successive fruiting branches and the appearance of successive squares, as determined by other investigators, show a close similarity to the intervals between blooms in a vertical and horizontal direction as indicated in the present paper.

A cyclic tendency in blooming in grouped plants is due to the tendency toward regularity in the individual plants. That the breaks in the flowering curves are not regular and pronounced is due (1) to conditions which upset the regularity in the individual plants, (2) to the fact that these plants begin blooming on different dates, and (3) to injuries and shedding, which to some extent modify results.

The horizontal interval of blooming varies on an average less than 0.4 from a 6-day interval and consistently increases at outer nodes of fruiting branches over the intervals found between nodes nearer the central stem. In other words, the early intervals are shorter than the later ones.

The average for vertical order of blooming is in very few instances outside the range 2.3 to 2.8 days. Usually the vertical interval decreased at the outer nodes, since blooms occurred at such nodes only on the earlier or lower fruiting branches and were a product of the vigorous growth period of the plants.

The ratio between the horizontal and vertical blooming intervals, though varying greatly, was in most instances between 2.6 and 2.1. To be strictly regular this ratio, it would appear, must be exactly 2, but the vertical intervals have been unduly shortened, increasing the ratios.

A study of the data of all workers shows but slight differences in the rapidity of blooming of cotton, due to difference in species, variety, altitude, latitude, season, or various cultural practices. To make cotton "bloom fast" is almost impossible, but the number of blooms per plant or per row or per day can be increased by increasing the size of the plant. The plants that produce 75 to 100 blooms will show so-called faster rates of blooming than plants that produce 20 to 30 blooms.

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# A COMPARISON OF METHODS FOR DETERMINING THE VOLUME-WEIGHT OF SOILS<sup>1</sup>

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## INTRODUCTION

Since some of the soils of New Mexico are extremely variable in texture, structure, and stratification, they present many problems to the investigator who tries to determine their volume-weights.<sup>2</sup> Because of these varying conditions, several of the common methods used in making volume-weight determinations can not be depended upon to give satisfactory results. Therefore, experimental work was undertaken to determine the most suitable method for use under these conditions. The paraffin-immersion method,<sup>3</sup> being impractical here, was not considered in these tests. Comparisons were made between the 1-foot cylinder method, the rubber-tube method,<sup>4</sup> the viscous-fluid method,<sup>5</sup> the improved soil-tube method,<sup>6</sup> and a revised sand method.<sup>7</sup>

## EQUIPMENT AND PROCEDURE

The cylinder used in the tests with the 1-foot cylinder method was made from a piece of large water pipe. (Fig. 1.) A section 13.25 inches in length was remodeled in a machine shop so that the inside diameter was 3.61 inches and the cylinder was uniform and smooth throughout.

Approximately one thirty-second inch was cut away from the outside of the cylinder from the top to within 1 inch of the lower end. From 1 inch downward the cylinder was beveled to a cutting edge, thus making it flush with the inside diameter and leaving a small flange on the outside to reduce friction. A driving plug was cut from a solid piece of material so that it was about 1.25 inches in thickness and of the same diameter as the outside of the cylinder. A seat about 0.2 inch in depth (it should have been deeper, so the plug would have remained in position better) was cut out on the lower side of the plug so that it seated snugly on top of and partially inside of

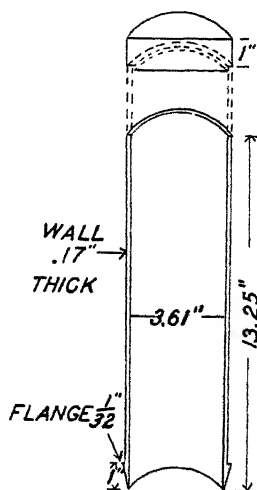


FIGURE 1.—Longitudinal section of cylinder and driving plug, with the approximate dimensions, used in the 1-foot cylinder method of soil volume-weight determination

<sup>1</sup> Received for publication Jan. 13, 1931; issued June, 1931.

<sup>2</sup> The volume-weight, or apparent specific gravity, of a soil is a figure which represents the ratio between the weight of a given volume of undisturbed water-free soil and the weight of an equal volume of water.

<sup>3</sup> SHAW, C. F. A METHOD FOR DETERMINING THE VOLUME WEIGHT OF SOIL IN FIELD CONDITION. Jour. Amer. Soc. Agron. 9: 38-42. 1917.

<sup>4</sup> ISRAELSEN, O. W. STUDIES ON CAPACITIES OF SOILS FOR IRRIGATION WATER, AND ON A NEW METHOD OF DETERMINING VOLUME WEIGHTS. Jour. Agr. Research 13: 28. 1918.

<sup>5</sup> BECKETT, S. H. THE USE OF HIGHLY VISCOUS FLUIDS IN THE DETERMINATION OF VOLUME-WEIGHT OF SOILS. Soil Sci. 25: 481-483. 1928.

<sup>6</sup> VEHRMEYER, F. J. AN IMPROVED SOIL-SAMPLING TUBE. Soil Sci. 27: 147. 1929.

<sup>7</sup> FREAR, W., and ERB, E. S., EXCAVATION METHOD FOR DETERMINING THE APPARENT SPECIFIC GRAVITY OF SOILS. Jour. Assoc. Off. Agr. Chem 4: 10. 1920.

the cylinder. Owing to the use of this plug, the cylinder was not damaged by driving with a 2-pound hammer.

The rubber tube used in these trials was 61 inches in length and slightly more than 1.5 inches in diameter. Rubber bandages, such as are commonly used by the medical profession, were made up into a tube of the desired dimensions by a local tire vulcanizer. This rubber seems to have been slightly stiff for this purpose. From a specific-gravity test on the rubber, it was found that 86 ml. of water were displaced by the tube.

The oil used with the viscous-fluid method is commonly known as "600 W." (Preliminary tests were made with a heavy road oil, but it penetrated so rapidly into the sandy strata that an accurate measurement could not be secured.) The volumes were obtained by weighing the oil used for each hole. This weight was multiplied by 1.1287 as a correction factor, which was obtained by dividing 100 by 88.6 to get the correct volume. A test on the oil showed that 100 ml. weighed 88.6 gm.

The tube used with the improved soil-tube method was like the one described by Veihmeyer. He stated that this tube was used with satisfactory results in making volume-weight determinations.

When the sand method was used the volume of the hole was determined by measuring volumetrically the sand required to fill the hole which had been previously drilled with an auger. The sand was obtained from a gravel pit and screened. Only the particles which would pass through a 2-mm. sieve and which were retained on a 1-mm. sieve were used. Tests with various sized particles showed that this size gave the most consistent results, so far as pouring and compaction were concerned. As a preliminary test an old corroded water pipe, which was sufficiently rough to resemble the wall of a hole in the soil, about 1.5 inches in diameter and 5 feet in length, was placed upright and filled with the sand, which was poured continuously from a 500-ml. graduated cylinder through a funnel with an opening of about three-eighths of an inch. The same funnel was used to fill the cylinder with sand. An effort was made to pour the sand into the cylinder in a continuous and uniform stream until it was almost full. The remaining portion was filled by hand, extreme care being used to prevent jarring, as the least blow would have caused the sand to settle. Repeated trials showed that the milliliters of sand used in filling the pipe multiplied by 0.9501 gave the correct volume, within reasonable limits, which had been previously determined with water. However, this factor was not used in making volume-weight calculations, because it was thought that the inside of the pipe was not exactly like the holes in the soil, so far as roughness was concerned. The factors used in the tests were calculated from the field work, that is, the total dry weight of the samples was divided by the average volume weight, as determined by the cylinder method, and this figure was divided by the total cubic centimeters of sand; the resulting figure was the correction factor. The number of cubic centimeters of sand for each sample was then multiplied by this factor to secure the volume weights of each sample.

The auger employed in making the holes when the rubber-tube method, the viscous-fluid method, and the sand method were used was made of a 1.5-inch wood bit welded to about 5 feet of  $\frac{1}{2}$ -inch

water pipe with a T joint at the top and with 8-inch pieces of pipe for a handle.

The soil in which tests were made was extremely variable in structure and texture and is classed as Gila clay adobe.<sup>8</sup> The surface soil for a depth of 18 to 20 inches was a clay adobe. Below this was a 4-inch layer of silty fine sand; then a layer about 6 inches in thickness composed of an intermingling of sand, silt, and adobe. Next was a 1-inch layer of fine tight clay, and then about 30 inches of material varying from adobe with accumulations of sand at the top to clean medium sand at the bottom. The change in texture from the top to the bottom of this layer was gradual.

To facilitate the making of these tests a trench was dug 2.5 feet wide, 6 feet deep, and about 20 feet long. Ten groups of tests were made along one side of this trench, all of which were included in an area about 18 feet long and 2.5 feet wide. Ten tests were made with the cylinder, 20 with the soil tube, 20 with sand, 20 with oil, and 20 with the rubber tube. The holes used for sand were first used for the

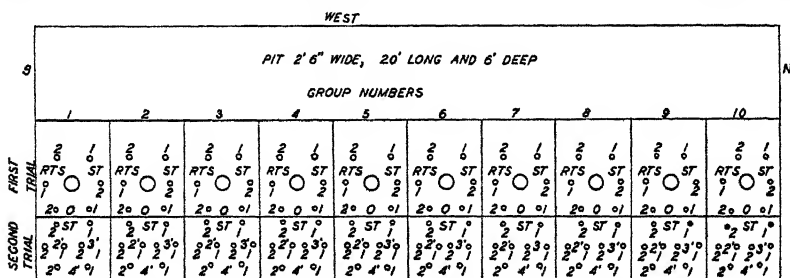


FIGURE 2.—Arrangement of pit, groups, and holes used in both trials. The sand method was used on the last two rows. See text for explanation

rubber-tube method. Figure 2 shows the arrangement of groups, holes, and pit.

The pit was dug so that as little soil as possible would be disturbed in taking the samples for the cylinder method. A large number of samples were taken in a very small area and theoretically there should have been very little difference in the soils from different groups. In the center of each group and about 18 inches from the edge of the pit a place was marked for the cylinder-method sample. On the east side of each group two holes were marked O for the oil method. On the northwest side two holes were marked ST for the soil-tube method, and on the southwest side two were marked RTS for the rubber-tube and sand methods. The holes in each group were from 4 to 6 inches apart and about 6 inches from the hole used for the cylinder method. The oil holes were placed on the far side from the pit so that the oil could be drained out after the cylinder-method sample had been taken and then used for the next set of holes. In doing this a small amount of sand accumulated in the oil, which probably changed its specific gravity and resulted in a lower volume-weight figure. However, this practice did not affect the spread of the results, since both the highest and lowest volume-weight figures were obtained from holes in which new oil was used.

The holes for the oil method, sand method, and rubber tube method were all made with the auger described above. The holes for the soil tube method were made with the improved soil tube. No

<sup>8</sup> NELSON, J. W., and HOLMES, L. C. SOILS SURVEY OF MESILLA VALLEY, NEW MEXICO-TEXAS. U. S. Dept. Agr., Bur. Soils Field Oper., 1912, Rpt. 14: 2011-2045, illus. 1915.

attempt was made to take any of these samples in foot sections. The soil tube was inserted in each hole from three to five times in taking the entire sample. When the cylinder method was used the samples were taken in 1-foot sections and the five weights totaled with the five volumes in making the volume-weight calculations. An attempt was made to take all samples for all methods to a depth of 60 inches, but with the cylinder method a slightly greater depth was obtained, although not enough to affect the results. The holes for the oil, soil tube, sand, and rubber-tube methods were made first, then the cylinder for the cylinder method was driven down 1 foot and excavated with a pick, chisel, saw, and shovel. It was found that very little if any compaction resulted from the use of the cylinder. Standard level marks were established with a stake about 2 feet from these holes, so that accurate measurements could be made on the depth of each sample. All of the soil taken from each hole was dried at about 105° C. and volume-weight calculations were made after the necessary correction factors had been applied. Table 1 shows the volume weights obtained by the different methods.

### EXPERIMENTAL DATA

The results obtained by the cylinder method are more uniform and consistent than those obtained by the other methods and it is believed that they are accurate and reliable; therefore, they are used as the standard by which the results from the other methods are compared with it as a standard.

TABLE 1.—*Individual soil volume-weight determinations made by different methods*

[First group of tests]

Group No.	Hole No.	Soil volume weight as determined by method indicated					
		Cylinder	Sand	Improved soil tube		Rubber tube	Viscous fluid
				First trial	Second trial		
1-----	1	1.45	1.48	1.28	1.33	1.35	1.05
1-----	2	1.45	1.48	1.38	1.38	1.39	1.86
2-----	1	1.45	1.26	1.44	1.40	1.20	1.19
2-----	2	1.39	1.39	1.43	1.38	1.29	1.05
3-----	1	1.42	1.51	1.43	1.42	1.40	1.33
3-----	2	1.45	1.49	1.38	1.39	1.37	1.23
4-----	1	1.45	1.40	1.39	1.39	1.35	1.32
4-----	2	1.45	1.45	1.30	1.37	1.38	1.25
5-----	1	1.45	1.46	1.43	1.42	1.39	1.26
5-----	2	1.44	1.47	1.41	1.40	1.38	1.21
6-----	1	1.44	1.45	1.35	1.40	1.36	1.27
6-----	2	1.42	1.40	1.42	1.41	1.40	1.61
7-----	1	1.42	1.45	1.37	1.28	1.37	1.26
7-----	2	1.41	1.48	1.23	1.17	1.30	1.29
8-----	1	1.41	1.39	1.36	1.34	1.30	1.80
8-----	2	1.39	1.44	1.35	1.34	1.33	1.24
9-----	1	1.39	1.42	1.33	1.38	1.34	1.28
9-----	2	1.43	1.38	1.38	1.35	1.35	1.28
10-----	1	1.39	1.38	1.38	1.32	1.29	1.61
10-----	2	1.44	1.44	1.36	1.34	1.36	1.61
Average-----		1.43±0.005	1.43±0.008	1.37±0.008	1.36±0.008	1.34±0.007	1.28±0.023

These tests were located, unavoidably, at right angles to and a short distance from a row of young shade trees. In making these determinations it was noticed that the roots increased in number from the first to the last group, and it is probable that the decrease

shown in the volume weights for the cylinder method is due to this factor. However, this condition was not so noticeable where the other methods were used, probably because the smaller instruments had a tendency to spread the roots rather than cut them off. Also a little more variation might have been secured if 20 determinations had been made instead of 10.

The determinations by the sand method, because of the correction factor used, gave the same average result as the cylinder method, but more variation was in evidence. The results obtained by the improved soil-tube method, shown in the fifth and sixth columns of Table 1, are about as variable as those obtained by the sand method, but are lower than those secured both by the cylinder and the sand methods. The results from the improved soil-tube method, shown in the fifth column, were secured from the first trial. The variation is about the same as for the sand method, but the average volume weight is lower than for the cylinder method. In order to check these data a second test was made, the results of which are shown in the sixth column. The average results for the second trial were practically the same as for the first and the variation was practically the same. This method is convenient and rapid, and it is probable that were the results multiplied by 1.0476, an accurate value would be secured. The results from the rubber-tube method were about the same as for the improved soil-tube method, but lower than for the cylinder method, and showed considerably more variation. Apparently a correction factor should be used in connection with this method to secure reliable results. The viscous-fluid method (where 600 W. oil was used) gave a low average volume weight with considerable variation in the individual determinations, and for this reason can not be recommended for use under such conditions as prevailed in these experiments.

Only half as many determinations were made by the cylinder method as by the others, and it would hardly be proper to compare 10 cylinder-method tests with 20 for the other methods. It was for this reason that Table 2 was prepared. Section A of this table shows the average volume weights, standard deviation, coefficient of variation, highest volume weight, lowest volume weight, and spread for all determinations for the various methods. Section B shows the same comparisons as section A, except that a summary of data from holes numbered 1 is used. In section C a summary of data from holes numbered 2 from all except the cylinder method was used. The first column of figures in the three sections of this table is the same, as duplicate holes were not used in connection with this method. A glance at section A shows that the cylinder method gave more satisfactory results than the others and that the viscous-fluid method gave the least reliable results. In section B, where only 10 determinations for each method were considered, the cylinder method also appears reliable and satisfactory, as compared with the other methods. The viscous-fluid method appears to be the least reliable, since the average volume-weight figure is very low and the spread, standard deviation, and coefficient of variation are all higher than for the other methods. The sand method, the improved soil-tube method, and the rubber-tube method are about the same so far as standard deviation and coefficient of variation are concerned.



The sand method presents more spread in the data than either of the other two in this section. In section C the same comparisons are made. The sand method is found to present less spread than the improved soil-tube method and it also shows a very small coefficient of variation. The viscous-fluid method appears to be very unsatisfactory in this section, because of the lack of consistency in the data.

TABLE 2.—Comparisons of results obtained in soil determinations using various volume-weight methods

SECTION A

	Values * given by the method indicated					
	3.61-inch cylinder	Sand	Improved soil tube		Rubber tube	Viscous fluid
			First trial	Second trial		
Trials, number.....	10	20	20	20	20	20
Average volume weights.....	1.43±0.005	1.43±0.008	1.37±0.008	1.36±0.008	1.34±0.007	1.28±0.023
Standard deviation.....	.0235	.0557	.0529	.0566	.0480	.1520
Coefficient of variation, per cent.....	1.64	3.90	3.86	4.16	3.58	11.95
Highest volume weight.....	1.45	1.51	1.44	1.42	1.40	1.86
Lowest volume weight.....	1.39	1.26	1.23	1.17	1.20	1.05
Spread.....	.06	.25	.21	.25	.20	.81

SECTION B

	10 (No. 1)	10 (No. 1)	10 (No. 1)	10 (No. 1)	10 (No. 1)	10 (No. 1)
Trials, number.....	10	10	10	10	10	10
Average volume weights.....	1.43±0.005	1.42±0.010	1.38±0.010	1.37±0.010	1.34±0.012	1.26±0.017
Standard deviation.....	.0235	.0469	.0480	.0447	.0557	.0787
Coefficient of variation, per cent.....	1.64	3.30	3.48	3.26	4.16	6.25
Highest volume weight.....	1.45	1.51	1.44	1.42	1.40	1.33
Lowest volume weight.....	1.39	1.26	1.28	1.28	1.20	1.05
Spread.....	.06	.25	.16	.14	.20	.28

SECTION C

	10 (No. 1)	10 (No. 2)	10 (No. 2)	10 (No. 2)	10 (No. 2)	10 (No. 2)
Trials, number.....	10	10	10	10	10	10
Average volume weights.....	1.43±0.005	1.44±0.006	1.36±0.012	1.35±0.014	1.36±0.008	1.30±0.042
Standard deviation.....	.0235	.0265	.0574	.0648	.0361	.1990
Coefficient of variation, per cent.....	1.64	1.84	4.22	4.80	2.65	15.31
Highest volume weight.....	1.45	1.49	1.43	1.41	1.40	1.86
Lowest volume weight.....	1.39	1.36	1.23	1.17	1.29	1.05
Spread.....	.06	.13	.20	.24	.11	.81

\* Formula used:

$$\text{Standard deviation, } SD = \sqrt{\frac{\sum d^2}{n}}$$

$$\text{Coefficient of variation, } CV = \frac{SD \times 100}{m} \quad d = \text{deviation; } n = \text{number of items; } m = \text{mean.}$$

$$\text{Probable error of mean} = \frac{0.6745 \times SD}{\sqrt{n}}$$

A comparison of the three sections of this table indicates that there is little, if any, difference between the improved soil-tube method, the sand method, and the rubber-tube method so far as consistency of the data is concerned; and apparently a correction factor should be applied, to secure correct results, where any one of these methods is used. Since sections B and C show some differences in the results from duplicate sets of holes, it appears that at least 10 determinations should be made on soils of this type to obtain reliable results.

After the tests described above were finished and the calculations made, it was thought wise to make further experiments with the sand method. Apparently this method is as accurate and reliable as any except the cylinder method, and it can be used in places where the improved soil-tube method is not practical. Therefore, additional tests for 2, 3, and 4 feet were made in order to determine the correction factor for these depths.

A comparison of the results from all tests with the sand and cylinder methods for the same depths is presented in Table 3. The correction factors shown were computed in the same manner as the correction factor described above. The factors for depths of 3, 4, and 5 feet are about the same, but the one for the first 2 feet is a little large, for no apparent reason.

TABLE 3.—*Individual soil volume-weight determinations made by cylinder and sand methods at various depths*

[Second group of tests]

Group No.	Hole No.	Volume weights, for—							
		First 2 feet by—		First 3 feet by—		First 4 feet by—		First 5 feet by— <sup>a</sup>	
		Cylinder method	Sand method	Cylinder method	Sand method	Cylinder method	Sand method	Cylinder method	Sand method
1	1	1.46	1.41	1.48	1.48	1.45	1.39	1.45	1.48
1	2		1.45		1.39		1.38		1.48
2	1	1.43	1.55	1.44	1.48	1.44	1.43	1.45	1.26
2	2		1.50		1.29		1.44		1.36
3	1	1.44	1.49	1.44	1.54	1.41	1.49	1.42	1.51
3	2		1.49		1.41		1.47		1.49
4	1	1.45	1.37	1.46	1.49	1.45	1.48	1.45	1.40
4	2		1.34		1.48		1.51		1.45
5	1	1.43	1.40	1.43	1.52	1.43	1.33	1.45	1.46
5	2		1.48		1.44		1.44		1.47
6	1	1.42	1.47	1.43	1.50	1.43	1.46	1.44	1.45
6	2		1.37		1.42		1.39		1.40
7	1	1.40	1.26	1.40	1.46	1.40	1.32	1.42	1.45
7	2		1.31		1.45		1.50		1.48
8	1	1.37	1.42	1.38	1.29	1.39	1.37	1.41	1.39
8	2		1.45		1.34		1.36		1.44
9	1	1.36	1.44	1.37	1.29	1.37	1.34	1.39	1.42
9	2		1.28		1.31		1.37		1.43
10	1	1.30	1.35	1.33	1.38	1.36	1.37	1.39	1.38
10	2		1.40		1.44		1.36		1.44
Average		1.41 ±0.010	<sup>b</sup> 1.41 ±0.011	1.42 ±0.009	<sup>b</sup> 1.42 ±0.012	1.41 ±0.007	<sup>b</sup> 1.41 ±0.009	1.43 ±0.005	<sup>b</sup> 1.43 ±0.008

<sup>a</sup> The figures in these two columns were taken from Table 1.

<sup>b</sup> The correction factor for the sand method is 0.9195 for the first 2 feet, 0.8800 for the first 3 feet, 0.8744 for the first 4 feet, and 0.8763 for the first 5 feet.

Comparisons of these data on the basis of 10 tests each are shown in Table 4 and divided into three sections as in Table 2. Table 4 shows that the results obtained by the cylinder method are more consistent than those obtained by the sand method. However, the results with the sand method in this trial are consistent with those in the first. Apparently this method can be used with a fair degree of accuracy for at least 5 feet in depth.

TABLE 4.—Comparisons between results obtained in soil volume-weight determinations for different depths, using the cylinder and sand methods

## SECTION A

	Values for the—							
	First 2 feet by—		First 3 feet by—		First 4 feet by—		First 5 feet by—	
	Cylinder method	Sand method	Cylinder method	Sand method	Cylinder method	Sand method	Cylinder method	Sand method
Trials, number.....	10	20	10	20	10	20	10	20
Average volume weight.....	1.41	1.41	1.42	1.42	1.41	1.41	1.43	1.43
Standard deviation.....	±0.010	±0.011	±0.009	±0.012	±0.007	±0.009	±0.005	±0.008
Coefficient of variation, per cent.....	.0471	.0755	.0434	.0781	.0303	.0583	.0235	.0557
Highest volume weight.....	3.34	5.35	3.06	5.50	2.18	4.13	1.64	3.90
Lowest volume weight.....	1.46	1.55	1.48	1.54	1.45	1.51	1.45	1.51
Spread.....	1.30	1.26	1.33	1.29	1.36	1.32	1.39	1.26
.....	.16	.29	.15	.25	.09	.19	.06	.25

## SECTION B

Trials, number.....	10 (No.1)	10 (No.1)	10 (No.1)	10 (No.1)	10 (No.1)	10 (No.1)	10 (No.1)	10 (No.1)
Average volume weight.....	1.41	1.42	1.42	1.44	1.41	1.40	1.43	1.42
Standard deviation.....	±0.010	±0.016	±0.009	±0.018	±0.007	±0.013	±0.005	±0.010
Coefficient of variation, per cent.....	.0471	.0782	.0434	.0866	.0303	.0609	.0235	.0469
Highest volume weight.....	3.34	5.37	3.06	6.01	2.18	4.29	1.64	3.30
Lowest volume weight.....	1.46	1.53	1.48	1.54	1.45	1.49	1.45	1.51
Spread.....	1.30	1.26	1.33	1.29	1.36	1.32	1.39	1.26
.....	.16	.29	.15	.25	.09	.17	.06	.25

## SECTION C

Trials, number.....	10 (No.1)	10 (No.2)	10 (No.1)	10 (No.2)	10 (No.1)	10 (No.2)	10 (No.1)	10 (No.2)
Average volume weight.....	1.41	1.41	1.42	1.40	1.41	1.42	1.43	1.44
Standard deviation.....	±0.010	±0.016	±0.009	±0.013	±0.007	±0.012	±0.005	±0.006
Coefficient of variation, per cent.....	.0471	.0745	.0434	.0600	.0303	.0545	.0235	.0265
Highest volume weight.....	3.34	5.30	3.06	4.29	2.18	3.86	1.64	1.84
Lowest volume weight.....	1.46	1.50	1.48	1.48	1.45	1.51	1.45	1.49
Spread.....	1.30	1.28	1.33	1.29	1.36	1.36	1.39	1.36
.....	.16	.22	.15	.19	.09	.15	.06	.13

## SUMMARY

This paper reports a comparative study of five different methods of determining soil-volume weights. These tests were made on Gila clay adobe to a depth of 5 feet, and the conclusions are based on the study of only this particular soil.

Ten determinations were made with the cylinder method, 20 with the viscous-fluid method, 20 with the rubber-tube method, 40 with the improved soil-tube method, and 80 with the sand method. In all, 170 determinations were made in an area about 20 by 2½ feet.

The cylinder method gave reliable and satisfactory results.

The viscous fluid method gave such variable results that it can not be recommended for such conditions as prevailed in these experiments.

The sand, rubber-tube, and improved soil-tube methods rank about the same so far as the variability of the results is concerned. To secure accurate results with these methods, a correction factor should be used.

# PERCENTAGE DRY MATTER AND FIELD WEIGHT OF EAR CORN FROM UNLIMED AND LIMED PLOTS<sup>1</sup>

By A. W. BLAIR

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In connection with the field studies on the availability of nitrogen that have been carried on at this station for the past 22 years, corn has been one of the crops in a 5-year rotation. The experiment provides for twenty  $\frac{1}{2}$ -acre plots with different nitrogen treatments, without lime, and twenty corresponding plots with lime. The lime material has been either ground oyster shells or ground limestone, and has been applied at the rate of 2 tons per acre once in five years. This has been sufficient to maintain the hydrogen-ion concentration of the soil at close to pH 6.8 to 7.0. The majority of the unlimed plots maintain a fairly constant pH value of about 5.2 to 5.3. A few, however, have a pH of 5.8 and one has a pH of 6.0.

The soil is a sassafras loam of fair quality. What treatment it received before the plots were laid out in 1908 is not known, but it is very probable that no lime had been used for many years.

Corn was grown in 1908, 1913, 1918, 1923, and 1928. The other crops in the rotation were oats, wheat, and for two years, timothy.

In preparing the samples for analysis, the percentage of dry matter in the ear corn was determined. The figures for 1918, 1923, and 1928, together with field weights of ear corn, were available and are shown in Table 1. In 1918 the corn from 18 of the 20 plots treated with lime showed a higher percentage of dry matter than the corn from plots without lime; in 1923 the corn from 16 of the 20 limed plots showed a higher percentage of dry matter than the corn from the corresponding unlimed plots, while in 1928 the corn from 17 of the 20 limed plots showed a higher percentage of dry matter than the corn from the corresponding unlimed plots. These differences are clearly brought out in the averages, the average difference being a little more than 2 per cent in 1918 and about 4 per cent in 1923 and 1928. This difference in content of dry matter in favor of the corn from the limed plots would seem to indicate that corn grown on acid soil does not mature and dry out quite so early as corn grown on the same soil with a fair supply of lime. Other experiments have shown that corn on very acid soil is inclined to remain green longer than that grown on well-limed soils. In the work reported here, however, it was only the corn on a few of the most acid plots that exhibited a difference that could be detected with the eye, but apparently the well-limed soil slightly speeded up maturity. Corn on these plots is usually harvested during the last half of September, and that date is not too early for maturity, if growing conditions have been favorable.

The field weight of ear corn is shown along with the percentage of dry matter. The general averages show some difference in favor of the limed plots. If, however, the weights for certain unlimed plots

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<sup>1</sup> Received for publication Dec. 19, 1930; issued June, 1931.

which have received extreme treatment should be omitted from the average, the difference between the yields from the limed and unlimed plots would be slight. It would appear, therefore, that in this case the effect of the lime has been in the direction of uniform ripening and drying of the corn rather than in increased weight. The unfavorable conditions which frequently exist in acid soils might account for the retarded maturity.

TABLE 1.—Field weight of ear corn (acre basis) and percentage dry matter in ear corn from unlimed and limed plots

Plot No	Fertilizer treatment (per acre)	Field weight and percentage dry matter in ear corn raised in—									
		1918				1923				1928	
		Without lime		With lime		Without lime		With lime		Without lime	
		Pounds	Per cent	Pounds	Per cent	Pounds	Per cent	Pounds	Per cent	Pounds	Per cent
1	Nothing.....	4,380	63.29	3,850	65.38	3,100	67.18	3,475	71.29	1,900	64.21
2	160 pounds muriate of potash.....	3,720	66.99	4,050	66.70	2,500	60.20	4,300	73.95	1,525	65.57
3	320 pounds superphosphate.....	4,140	67.54	3,790	69.90	3,650	64.32	3,250	72.15	1,750	68.29
4	Minerals only.....	3,360	67.02	3,720	70.54	3,225	65.66	4,000	72.25	1,550	66.13
5	Minerals and 16 tons cow manure.....	4,420	65.88	3,990	70.74	4,550	70.49	3,700	75.07	4,400	72.16
6	Minerals only.....	4,760	68.91	4,350	71.14	5,200	68.08	5,075	73.35	4,150	71.39
7	Nothing.....	2,840	68.38	3,910	69.84	1,350	63.70	3,750	71.07	826	69.51
8	Minerals and 160 pounds NaNO <sub>3</sub> .....	3,400	68.71	4,010	70.06	3,000	63.50	4,525	71.22	1,650	69.70
9	Minerals and 320 pounds NaNO <sub>3</sub> .....	4,120	69.81	3,970	68.35	3,625	69.86	3,800	69.67	2,600	70.67
10	Minerals and Ca(NO <sub>3</sub> ) <sub>2</sub> equivalent to 320 pounds NaNO <sub>3</sub> .....	4,020	66.27	4,700	70.04	3,800	62.70	5,000	72.25	2,350	69.36
11	Minerals and (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> equivalent to 320 pounds NaNO <sub>3</sub> .....	1,340	67.46	4,560	70.00	1,450	62.93	4,550	71.65	76	71.05
12	Minerals and CaCN <sub>2</sub> equivalent to 320 pounds NaNO <sub>3</sub> .....	4,320	68.61	3,880	70.62	4,425	68.87	5,000	69.55	2,625	76.67
13	Minerals only.....	4,450	66.61	3,820	69.95	4,650	66.45	4,900	70.31	900	69.44
14	Minerals and NaNO <sub>3</sub> (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (N, half and half).....	4,500	68.98	3,920	70.41	4,875	71.74	4,600	74.24	2,900	66.81
15	Minerals and tankage equivalent to 320 pounds NaNO <sub>3</sub> .....	3,900	67.90	4,080	73.04	3,825	74.58	4,425	73.33	3,350	68.06
16	Minerals only.....	4,820	70.04	4,140	70.24	4,325	74.62	4,525	75.86	2,750	68.13
17	do.....	3,300	68.36	5,000	72.56	2,125	71.76	4,325	72.25	1,125	72.22
18	Minerals, 16 tons manure, and 320 pounds NaNO <sub>3</sub> .....	4,720	70.25	5,120	70.94	5,150	72.14	5,550	71.76	5,175	74.88
19	Minerals only.....	3,040	69.34	4,180	73.17	2,675	72.15	3,825	73.79	1,325	67.92
20	Minerals, 2 tons rye straw, and 320 pounds NaNO <sub>3</sub> .....	4,610	68.23	4,870	70.03	4,525	70.99	3,825	65.03	2,008	74.71
	Average.....	3,885	67.93	4,195	70.18	3,601	68.10	4,320	72.00	2,186	69.84
										2,571	73.67

\* Minerals=160 pounds muriate of potash and 320 pounds superphosphate.

In the eastern part of the United States there are millions of acres of land that have not been limed in many years, and it seems entirely possible that in the case of much of this land, acidity may have become one of the limiting factors in the successful growing of corn as well as of many other crops.

### SUMMARY

Analyses of ear corn grown on sassafras loam of fair quality, fertilized in different ways, indicate that corn grown on acid soil does not mature and dry out quite so early as corn grown on the same soil with a fair supply of lime. General averages also indicate some difference in favor of the limed plots when the field weight of ear corn is considered, but if the weights for certain unlimed plots that received extreme treatment are omitted from the averages, the differences in yields between the limed and the unlimed plots are slight. Figures for corn grown in 1918, 1923, and 1928 are summarized.

# CORRELATED INHERITANCE IN A CROSS (SEVIER $\times$ DICKLOW) $\times$ DICKLOW WHEATS<sup>1</sup>

By GEORGE STEWART, *Agronomist*, and R. K. BISCHOFF, *Graduate Student*,  
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## INTRODUCTION

This paper reports a study of the inheritance, particularly the correlated inheritance, of certain observed and measured plant characters in a wheat cross between a segregate from Dicklow  $\times$  Sevier (F22), and a pure line from Dicklow (D3), one of the original parents of F22.

Since Hayes and Garber (4),<sup>2</sup> Clark (1), and Stewart (6) have recently compiled bibliographies of literature relating to the inheritance of the characters in wheat herein reported, literature citations are limited to those that refer directly to the material studied.

## DESCRIPTION OF PARENTS

F22, true breeding for the characters studied, is a segregate from the cross Sevier  $\times$  Dicklow, developed at the Utah station. The straw is much stronger than that of the Sevier parent, but not so strong as that of the Dicklow parent. The glumes are light bronze in color. It is a fully awned wheat, with awns 60 to 70 mm. long.

Measurements of the parent rows of F22 are summarized as follows:

Length of longest culm to base of spike.....	108. 43 $\pm$ 2. 06 cm.
Spike density—length of 10 rachis internodes.....	25. 61 $\pm$ . 86
Number of culms per plant.....	11. 61 $\pm$ . 84

Dicklow is a favorite spring wheat on the irrigated farms of Utah. Its popularity is due to its ability to resist lodging under irrigation and to produce a high yield. It has a medium tall, stiff straw. The lemmas near the apex of the spike bear short awns from 3 or 4 to 10 mm. in length. The glumes are white.

Measurements of the Dicklow parent rows follow:

Length of longest culm to base of spike.....	103. 84 $\pm$ 1. 80 cm.
Spike density—length of 10 rachis internodes.....	50. 3 $\pm$ . 577
Number of culms per plant.....	9. 43 $\pm$ 1. 46

These measurements indicate that F22 has a culm length averaging  $4.59 \pm 2.74$  cm. longer than Dicklow. The difference is less than twice the probable error. The range for the longest culm is 92.5 to 123 cm. for F22, and from 93.5 to 117.4 for Dicklow. In spike density Dicklow, ranging from 43.47 to 64.75 cm. for 10 internodes, is nearly twice as lax as the F22 parent whose range is from 23.83 to 28.05 cm. The two parents differ but slightly in number of culms produced. F22 produces a mean of  $11.61 \pm 0.84$  culms, with a range from 9.3 to 15.7 per plant. Since the Dicklow parent has a mean of  $9.43 \pm 1.46$  ranging from 7.7 to 11.4 per plant, there is no real difference between the parents in this respect.

<sup>1</sup> Received for publication Nov. 7, 1930; issued June, 1931. Contribution from the Department of Agronomy, Utah Agricultural Experiment Station.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 790.

## EXPERIMENTAL PROCEDURE

The cross between a pure strain of F22 and one of Dicklow (D3) was made in 1925 at Logan, Utah. The  $F_1$  plants were grown in 1926 and the  $F_2$  families in 1927. One of the most vigorous of the  $F_2$  families was chosen to continue the study. This family consisted of 257 plants, of which 51 were dwarfs. Of the 51 dwarfs 11 produced either no seed or not enough with which to propagate  $F_3$  progenies. Each plant, whose development permitted it, was classified according to awn class, glume color, and spike density. The spike-density figure was obtained by measuring the length of 10 rachis internodes. Grain from each  $F_2$  plant seeded an  $F_2$  progeny row in 1928. The kernels were spaced about 3 inches apart, with 40 to 60 seeded in a row except where the  $F_2$  plant furnished fewer kernels. The rows were 1 foot apart.

After each tenth progeny row the parental varieties, F22 and Dicklow, were sown side by side in the same manner and at the same time as the progeny rows. In all, there were 25 parent rows. Two of the Dicklow parent rows were destroyed by birds and are not included in the calculations. This planting arrangement made it possible to study the progeny characters in relation to the parental characters. One progeny row was also destroyed by birds.

When the grain was mature each  $F_3$  progeny and each parent row was carefully harvested by pulling the plants individually. The plants of each row were bundled together, tied, and labeled. The material was worked in the laboratory during the winter months, except that awn data were taken in the field for the progenies with true-breeding awns. Culm length for the 237 rows in which there were 10 or more normal plants was measured by placing the root end of the individual plant against a footboard and extending the culms along a table board marked in centimeters. The longest culm was measured to the base of the spike and recorded. The number of dwarfs in all of the 245 progenies was determined by counting, which, with 11  $F_2$  dwarfs without progenies, makes 256 for  $F_2$  plants classified for this character. In all other cases the 237 progeny rows with 10 or more normal plants were used except for awns, the data for which were secured on one additional progeny later destroyed by birds. The culms were counted with the precaution of avoiding second-growth culms; these frequently appeared at the base of the plant. Each plant was examined for its awn characteristics. Those that approached the F22 parent as determined by visual comparison with parent plants were classified as belonging to awn class 4, or simply to "awns 4." Those that approached the Dicklow parent were called "awns 2." Progenies that produced both awns-2 plants and awns-4 plants were said to be segregating. Spike density was determined by measuring 10 rachis internodes in the middle of a typical spike on each plant. This avoided the extremes of laxity and density found on opposite ends of the spike. Each plant was classified for glume color as white or bronze, determined by inspection.

The data were so taken and recorded that all data from each plant could be traced readily to that plant and to the row from which it came. This permitted the study of correlations.

The usual number of plants in each progeny was 40 to 45. In some progenies there were fewer than this, and in others there were more.

The parental rows consisted of approximately the same number of plants as did the progeny rows. No theory of inheritance was considered until all data were taken, recorded, and studied.

After the data were assembled and recorded, classifications and calculations were made. The mean length of the longest culm of all plants not classified as dwarfs was calculated for each progeny whether true breeding or not. The same calculation was made for the number of culms. The progeny rows were classified by inspection into segregating and true-breeding groups for stature, for awns, and for glume color. The mean spike density of each progeny row and of each parental row was obtained as well as the coefficient of variability (C. V.) for each row. When correlation studies were made the mean values for the  $F_3$  progenies were the figures used. In other words, the  $F_2$  plants were classified by the breeding behavior of their respective  $F_3$  progenies. This was regarded as a more accurate indication of the genetic composition of the  $F_2$  plants than was the individual character of the  $F_2$  plants themselves.

## EXPERIMENTAL RESULTS AND THEIR INTERPRETATION

Inheritance studies were made of individual plant characters, and correlation studies were made between various pairs of characters.

## INHERITANCE OF INDIVIDUAL CHARACTERS

Inheritance studies were made for the following individual plant characters: Culm length, stature, number of culms, awn classes, spike density, and color of glume.

TABLE 1.—Parental rows and  $F_3$  progenies arranged according to means of culm length and according to the coefficients-of-variability (C. V.) classes

[Cross 35c; grown in 1928 at Logan, Utah. Length of longest culm in centimeters, normal plants]

[illegible]



## CULM LENGTH

In Table 1 are compared the distribution of normal plants in the  $F_3$  progenies having 10 or more normal plants for culm length and its respective coefficient of variability, and the same data for the parental rows. The range for  $F_{22}$  parents is from 92.5 to  $123 \pm 6.06$  cm.; that of the Dicklow parent from 93.5 to  $117.4 \pm 4.80$  cm. The ranges and the mean heights of the two parents are similar. The range in culm length from the  $F_3$  progenies (fig. 1) is from 88.0 to  $128.0 \pm 5.77$

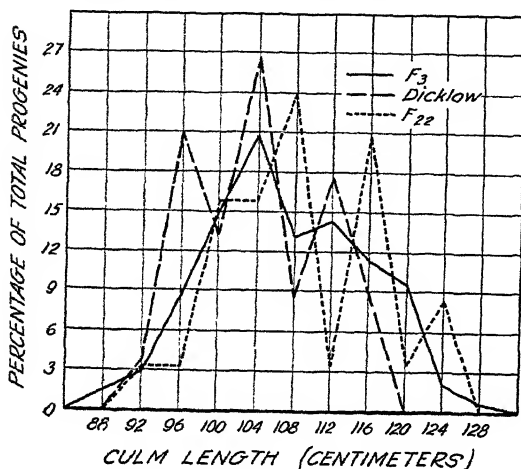


FIGURE 1.—Culm lengths of the two parents,  $F_{22}$  and Dicklow, and of the normal plants in all the  $F_3$  progenies. This character was widely variable, due to soil heterogeneity. After allowance is made for this variability there is no measurable difference between the two parents or between the parents and the  $F_3$  progenies.

cm. There were about 10 times as many individuals considered in the progenies as there were in the parental rows of each parent. The distribution shown fails to indicate segregating groups.

## STATURE

The  $F_3$  progenies segregated for normal and dwarf stature as follows: 98 were segregating for either 3 normal to 1 dwarf or 13 normal to 3 dwarf plants; 29 progenies were segregating 1 normal to 3 dwarf plants; 11 progenies were homozygous for the dwarf character, and 107 were homozygous for normal stature. Both the number of  $F_2$  plants and the breeding behavior of the  $F_3$  progenies suggest a 13:3 ratio for normal and dwarf segregation in  $F_2$ .

Two factors are involved in this explanation of dwarfness: (1)  $Ii$ , an inhibitor factor which prevents the expression of dwarfness when the dominant  $I$  is present either in the heterozygous or in the homozygous condition; and (2)  $Dd$ , a dwarf factor assumed to be a dominant, which produces dwarfs when in either the homozygous or the heterozygous condition unless the inhibiting factor is present as  $Ii$ .

The recombination of these factors would produce all normal plants in  $F_1$  but would call for two phenotypes in  $F_2$ , normal and dwarf, whose  $F_3$  characters and whose  $F_3$  breeding behavior might be summarized as follows:

- $P_0$   $F_{22}$   $IIDD \times$  Dicklow  $iidd$ .  
 $F_1$   $IiDd$ .  
 $F_2$  13 normal: 3 dwarf.

The 13 normal  $F_2$  plants would have the following genotypic composition and  $F_3$  breeding behavior:

- 1 *IIDD* (normal in  $F_2$ , normal in  $F_3$ ).
- 2 *IiDd* (normal in  $F_2$ , normal in  $F_3$ ).
- 2 *IiDD* (normal in  $F_2$ , segregate 3 normal:1 dwarf in  $F_3$ ).
- 4 *IiDd* (normal in  $F_2$ , segregate 13 normal:3 dwarf in  $F_3$ ).
- 1 *Iidd* (normal in  $F_2$ , normal in  $F_3$ ).
- 2 *Iidd* (normal in  $F_2$ , normal in  $F_3$ ).
- 1 *iidd* (normal in  $F_2$ , normal in  $F_3$ ).

The three dwarf  $F_2$  plants would have the following genotypic composition and  $F_3$  breeding behavior:

- 1 *iiDD* (dwarf in  $F_2$ , dwarf in  $F_3$ ).
- 2 *iiDd* (dwarf in  $F_2$ , segregate 3 dwarf: 1 normal in  $F_3$ ).

TABLE 2.—Segregation for normal and dwarf plants in  $F_3$  progenies from normal  $F_2$  plants, grouped into three classes determined by the nature of the segregation of normal to dwarf plants: 13:3, 3:1, and 1:3

[Family 35c, grown in 1928 at Logan, Utah]

CALCULATED SEGREGATION BASED ON 13:3

Normal plants	Dwarfs		Deviation calculated	P. E.	Dev / P. E.
	Found	Calculated			
Number	Number	Number			
27	5	6	1	1.49	0.67
38	4	7.9	3.9	1.71	2.27
31	4	6.6	2.6	1.56	1.66
31	5	6.8	1.8	1.58	1.13
40	1	7.7	6.7	1.69	3.96
25	1	4.9	3.9	1.34	2.91
32	3	6.7	3.7	1.56	2.36
34	7	7.7	.7	1.69	.41
23	3	4.9	1.9	1.34	1.41
44	6	9.4	3.4	1.86	1.83
34	5	7.3	2.3	1.64	1.40
37	6	8.06	2.06	1.73	1.19
27	1	5.3	4.3	1.39	3.09
36	6	7.9	1.9	1.71	1.11
31	5	6.8	1.8	1.58	1.13
32	2	6.4	4.4	1.54	2.85
24	3	5.01	2.01	1.37	1.46
40	6	8.6	2.6	1.79	1.45
40	3	8.06	5.06	1.73	2.92
36	5	7.7	2.7	1.69	1.59
25	3	5.3	2.3	1.39	1.65
29	2	5.8	3.8	1.47	2.58
37	7	8.3	1.3	1.75	.74
27	6	6.2	.2	1.51	.13
32	5	6.9	1.9	1.60	1.18
38	5	8.1	3.1	1.73	1.79
33	8	7.7	.3	1.69	.17
40	3	8.1	5.1	1.73	2.94
33	4	6.9	2.9	1.60	1.81
34	7	7.7	.7	1.69	.41
40	3	8.1	5.1	1.73	2.94
27	3	5.6	2.6	1.44	1.80
35	5	7.5	2.5	1.66	1.50
25	5	5.6	.6	1.44	.41
37	9	8.6	.4	1.79	.22
37	9	8.6	.4	1.79	.22
40	14	10.1	3.9	1.93	2.02
28	6	6.4	.4	1.54	.25
40	10	9.4	.6	1.86	.32
27	7	6.4	.6	1.54	.38
22	6	5.3	.7	1.55	.45
35	8	8.1	.1	1.73	.06
40	10	9.4	.6	1.86	.32
29	9	7.1	1.9	1.62	1.17
34	8	7.9	.1	1.71	.06
40	11	9.6	1.4	1.88	.74
40	8	9.0	1.0	1.82	.54
36	8	8.3	.3	1.75	.17
25	6	5.8	.2	1.47	.14
40	10	9.4	.6	1.86	.32
32	8	7.5	.5	1.66	.30
40	9	9.2	.2	1.64	.12
44	14	10.9	.5	2.15	.23

TABLE 2.—Segregation for normal and dwarf plants in  $F_3$  progenies from normal  $F_2$  plants, grouped into three classes determined by the nature of the segregation of normal to dwarf plants: 13:3, 3:1, and 1:3—Continued

## CALCULATED SEGREGATION BASED ON 3:1

Normal plants	Dwarfs		Devia- tion cal- culated	P. E.	Dev./P. E.
	Found	Calcu- lated			
Number	Number	Number			
33	10	10.8	0.8	1.92	0.41
34	13	11.8	1.2	2.00	.60
34	14	12.0	2.0	2.02	.99
33	15	12.0	3.0	2.02	1.48
27	16	10.8	5.2	1.92	2.70
24	10	8.5	1.5	1.70	.88
32	10	10.5	.5	1.89	.26
38	11	12.3	1.3	2.04	.63
28	17	11.3	5.7	1.96	2.90
38	13	12.8	.2	2.09	.09
28	16	11.0	5.0	1.94	2.57
34	14	12.0	2.0	2.02	.99
37	11	12.0	1.0	2.02	.49
29	11	10.0	1.0	1.85	.54
40	15	13.8	1.2	2.17	.55
31	13	11.0	2.0	1.94	1.08
28	13	10.3	2.7	1.87	1.44
31	9	10.0	1.0	1.85	.54
27	12	9.8	2.2	1.82	1.20
27	10	9.3	.7	1.78	.39
20	8	7.0	1.0	1.55	.64
31	16	11.8	4.2	2.00	2.10
37	11	12.0	1.0	2.02	.49
27	14	10.3	3.7	1.87	1.97
19	9	7.0	2.0	1.55	1.29
29	10	9.8	.2	1.82	.10
24	13	9.3	3.7	1.78	2.07
35	10	11.3	1.3	1.96	.66
29	13	10.5	2.5	1.89	1.32
24	7	7.8	.8	1.63	.49
23	7	7.5	.5	1.60	.31
36	12	12.0	.0	2.02	.00
26	10	9.0	1.0	1.75	.57
38	14	13.0	1.0	2.11	.47
40	14	13.5	.5	2.15	.23
22	8	7.5	.5	1.60	.31
29	12	10.3	1.7	1.87	.90
25	11	9.0	2.0	1.75	1.15
24	10	8.5	1.5	1.70	.88
37	12	12.3	.3	2.04	.15
39	14	13.25	.8	2.13	.37
27	13	10.0	3.0	1.85	1.62
19	7	6.5	.5	1.49	.33
29	11	10.0	1.0	1.85	.54
27	15	10.5	4.5	1.89	2.38

## CALCULATED SEGREGATION BASED ON 1:3

15	25	30.0	5.0	1.85	2.70
15	16	23.2	7.2	1.63	4.42
19	25	33.0	8.0	1.94	4.12
12	31	32.2	1.2	1.92	.62
15	36	38.2	2.2	2.09	1.05
7	23	22.5	.5	1.60	.31
22	30	39.0	9.0	2.11	4.27
11	38	36.7	1.3	2.04	.64
18	33	38.2	5.2	2.09	2.49
14	24	28.5	4.5	1.80	2.50
10	26	27.0	1.0	1.75	.57
10	25	26.2	1.2	1.73	.69
9	30	29.2	.8	1.82	.44
12	42	40.5	1.5	2.15	.70
15	26	30.7	4.7	1.87	2.51
12	34	34.5	.5	1.98	.25
14	36	37.5	1.5	2.07	.72
14	37	38.2	1.2	2.09	.57
10	34	33.0	1.0	1.94	.52
9	24	24.7	1.7	1.68	.42
10	37	35.2	1.8	2.00	.90
7	40	35.2	4.8	2.00	2.40
25	35	45.0	10.0	2.28	4.42
16	38	40.5	2.5	2.15	1.16
13	32	33.7	1.7	1.96	.87
13	25	28.5	3.5	1.80	1.94
5	31	27.0	4.0	1.75	2.29
28	42	52.5	10.5	2.44	4.80
7	33	30.0	3.0	1.85	1.62

It was impossible by inspection to separate the 13:3 and the 3:1 segregating groups; therefore, the separation was attempted by the Dev./P. E. method and is summarized in Table 2. The Dev./P. E. calculation was made for each progeny considered on the basis of a 13:3 segregation and also on the basis of a 3:1 segregation. The group segregating 1 normal:3 dwarf was also included in the calculation. The final separation was made on the basis of the smallest Dev./P. E. calculation. By this calculation 45 progenies were found to be segregating for the 3:1 ratio; 53 for the 13:3 ratio; and 29 for the 1:3 ratio. There were 11 progenies homozygous for dwarf stature and 107 homozygous for normal stature. In addition, there were 11  $F_2$  dwarfs which produced no  $F_3$  progenies. Forty other phenotypically similar  $F_2$  plants of dwarf stature produced 29 progenies segregating 1 normal:3 dwarf, and 11 progenies breeding true for dwarf stature. If the 11  $F_2$  dwarfs without progeny be assumed to segregate in the same proportions, this would mean that 3 of the 11 would have been true-breeding for dwarf stature and that 8 would have segregated 1 normal:3 dwarf.

The Dev./P. E. method of separating progenies was effective in separating the 1:3 group from the other two. The 13:3 and the 3:1 groups were not satisfactorily separated, perhaps on account of the unequal number (twice as many in the 13:3 group) of overlapping variants from two groups so closely similar in the nature of their segregation. The closeness-of-fit study would have been more satisfactory had it been possible to make a complete separation between the 13:3 and the 3:1 segregating groups. Since, however, this could not be done with the data available, the two groups were left together. By this grouping there were 98 progenies segregating 13:3 or 3:1, and 29 progenies segregating 1:3 to which must be added 8, the proportionate share of the 11  $F_2$  dwarf plants which had no progeny, leaving 3 to be classified as true dwarf. This makes 37 progenies in the 1:3 group, and 14 in the true-breeding dwarf group (11 with true-breeding dwarf progenies plus 3 from the 11 with no progenies). In order to see how well the theory fits the facts,  $X^2$  and  $P$  were calculated in Tables 3 and 4. Table 3, with the 13:3 and the 3:1 groups split, shows a poor fit,  $P=0.08$ , but since all the calculated groups are close to the observed, save the 13:3 and the 3:1, Table 4 gives the closeness of fit with these two expected groups regarded as one, which in this case more nearly approximates the actual data.

TABLE 3.—Closeness of fit of five groups of progenies on a basis of 13:3  $F_2$  segregation

[Family 35c; grown in 1928 at Logan, Utah]

Group	Observed value (O)	Calculated value (C)	O-C	(O-C) <sup>2</sup>	$\frac{(O-C)^2}{C}$
Homozygous normal.....	107	112	-5	25	0.2232
Segregating 13:3.....	53	64	-11	121	1.8906
Segregating 3:1.....	45	32	13	169	5.2813
Segregating 1:3.....	37	32	5	25	.7813
Homozygous dwarf.....	14	16	-2	4	.2500

 $X^2=8.4264$ .  $P=0.0786$ .

TABLE 4.—*Closeness of fit of four groups of progenies on a basis of 13 : 3 F<sub>2</sub> segregation*  
 [Groups 13:3 and 3:1 not separated; family 35c, grown in 1928 at Logan, Utah]

Group	Observed value (O)	Calculated value (C)	O-C	(O-C) <sup>2</sup>	$\frac{(O-C)^2}{C}$
Homozygous normal.....	107	112	-5	25	0.2232
Segregating 13:3 and 3:1.....	98	96	2	4	.0416
Segregating 1:3.....	37	32	5	25	.7813
Homozygous dwarf.....	14	16	-2	4	.2500

$$\chi^2=1.2961. \quad P=0.7335.$$

As applied in Table 4, the theory fits the observed data so well that in 73 cases out of 100 a wider deviation might be expected, due to chance alone.

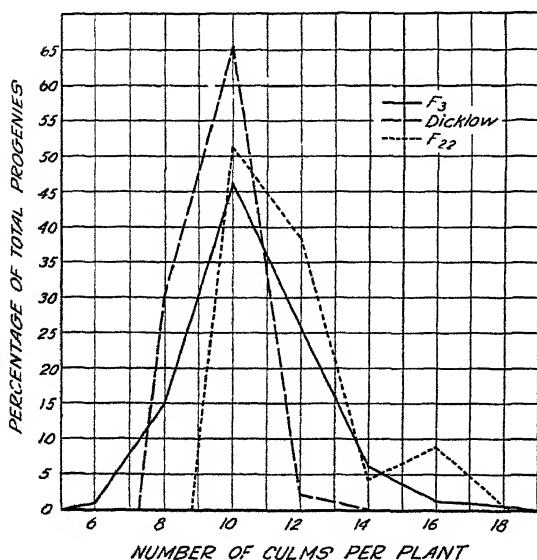


FIGURE 2.—Number of culms for the two parents, F<sub>22</sub> and Dicklow, and for the F<sub>3</sub> progenies. This character varied widely, due to soil heterogeneity. No measurable differences were found between the parents or between the parents and the F<sub>3</sub> progenies

$9.43 \pm 1.46$ , respectively. There seems to be some segregation, but the environmental variability as shown in Table 5 is so high as to hide its nature. The mean coefficients of variability are almost identical for both parents and for the F<sub>3</sub> progenies. Both parental types were recovered, as the curves in Figure 2 show.

The agreement of the theory with the data from the F<sub>2</sub> plants (expected dwarfs, 48.2; obtained, 51.0; deviation, 2.8; probable error, 4.22) and with the data from the F<sub>3</sub> progenies indicates that the theory of one dominant dwarfing factor *Dd* and an inhibitor *Ii* probably correctly explains the results obtained.

#### NUMBER OF CULMS

As judged by their probable errors, the F<sub>22</sub> and Dicklow parents are essentially alike in the number of culms produced, being  $11.61 \pm 0.84$  and

#### AWN CLASSES

In summarizing various inheritance studies, Hayes and Garber (4) found awn inheritance to be simple in crosses between some varieties and in others more complex. They concluded that there may be one or two or more genetic factors involved for awns. Howard and Howard (5) obtained single-factor results in some crosses, but in others between fully awned and awnless parents they were able to separate the F<sub>2</sub> progenies into five or six classes, which results required two factors to explain.

TABLE 5.—*Parental rows and F<sub>3</sub> progenies arranged according to number of culm classes and according to coefficient-of-variability (C. V.) classes*

[Cross 35c; grown in 1928 at Logan, Utah]

Parent or progeny	Number of progenies in culm class indicated																Total	C. V. classes
	6	7	8	9	10	11	12	13	14	15	16	17						
																	Number	Per cent
Dicklow	{	1	1	2	1												5	30
		2	3	3	3	1											9	40
		1	2	2	2												7	50
			1		1												2	60
Total or mean																	23	42.48
F22	{				2									1			3	30
					2	5	3	3			1						14	40
					1	2	1					1					5	50
				1		2										3	60	
Total or mean																	25	44.45
F3	{				1												2	20
			3	11	4	6	4			3	2						35	30
		2	3	15	25	24	20	8	1	2							106	40
			4	15	21	12	5	5	2	2	1		1	1			68	50
			3														19	60
				1	5	3	7	2	1								5	70
			1	1												2	80	
Total or mean																	237	43.96

In this cross, the F22 parent is homozygous for fully developed awns (awns 4), and the Dicklow parent for tip awns (awns 2). There were 56 progenies homozygous for awns 2, 131 were heterozygous for awn inheritance, and 51 were breeding true for awns 4. When compared for closeness of fit on the basis of a 1:2:1 segregation,  $\chi^2=2.6303$  and  $P=0.2766$ . (Table 6.) This fit indicates that the segregation considered is probably correct since a worse fit would be expected in 28 cases out of every 100 due to chance alone. No indication was found as to the dominance or recessiveness of awns.

TABLE 6.—*Closeness of fit of three groups of F<sub>3</sub> progenies on a basis of 1:2:1 segregation for awns*

[Family 35c; grown in 1928 at Logan, Utah]

Group	Observed value(O)	Calculated value(C)	O-C	(O-C) <sup>2</sup>	$\frac{(O-C)^2}{C}$
Homozygous awns 2.....	56	59.5	-3.5	12.25	0.2059
Heterozygous.....	131	119	12.0	144.00	1.2101
Homozygous awns 4.....	51	59.5	-8.5	72.25	1.2143

 $\chi^2=2.6303$ .  $P=0.2766$ .

## SPIKE DENSITY

The mean spike densities and the coefficients of variability were calculated for each progeny. The F<sub>3</sub> progenies were classified into three groups: (1) Those homozygous for dense spikes; (2) those heterozygous for spike density, and (3) those homozygous for lax spikes.

Table 7 compares the spike-density classes in their coefficient-of-variability (C. V.) classes of both parents and F<sub>3</sub> progenies.

TABLE 7.—Frequency distribution of the rows of the Dicklow and F22 parents and of F<sub>3</sub> progenies, arranged into classes according to the mean spike density and according to the coefficients-of-variability (C. V.) classes of the individual rows of parents and of the F<sub>3</sub> hybrid progenies

[Family 35c; grown in 1928 at Logan, Utah; millimeters for 10 rachis internodes]

Strain	Number of progenies of mean spike density indicated												Total	C. V. classes	
	20	24	28	32	36	40	44	48	52	56	60	64			
F22		3 15 1	6										Number 3 21 1	Per cent 10 14 18	
Total or mean													25	13.68	
Dicklow							1	4 5 1	8 2 1				12 7 3 1	8 12 16 20	
Total or mean													23	10.54	
Homozygous dense	1 12 6 1	1 17 16 1	3 1	2									2 29 27 1	6 10 14 18 22	
Total or mean													60	12.00	
Heterozygous		2 1 1 1	1 3 3 1 2	2 15 14 8 2	4 12 13 7 1	1 6 6 2 1	1 3 2	1 1					1 9 17 39 37 19 6	24 28 32 36 40 44 48	
Total or mean													128	38.15	
Homozygous lax								1 4 1	3 8 2	15 5 8	7 5 1	2 2 1	2 27 19 1	4 8 12 16	
Total or mean													49	9.04	

TABLE 8.—Frequency distribution of the F<sub>3</sub> progenies from a cross of Dicklow × F22, arranged into classes according to the mean spike density and according to coefficient-of-variability (C. V.) classes

[Three classes. (1) Those homozygous for dense spikes; (2) those heterozygous; and (3) those homozygous for lax spikes are plotted in one table to show the well-defined grouping of the three classes. Family 35c, grown in 1928 at Logan, Utah; spike density for 10 internodes in millimeters]

Number of F <sub>3</sub> progenies in mean spike density class indicated												Total	C. V. classes	
20	24	28	32	36	40	44	48	52	56	60	64			
1 12 6 1	1 17 16 1		1 1				1 4	3 8	15 5 1	2 7 2	1	Number 4 57 45 2 1 1 9 17 39 38 38 18 6	Per cent 6 10 14 18 22 26 30 34 38 42 46 50	
	2 1 1	1 3 3 2	2 15 14 8 2	4 12 14 7 1	1 6 6 2 1	1 2	1					237		

Heterozygosity or homozygosity was indicated by the size of the coefficient of variability. The mean coefficient of variability for the homozygous F22 parent was  $13.68 \pm 1.428$ , with a range from 10.24 to 16.25 per cent. The same calculation for Dicklow was  $10.54 \pm 1.018$ , with a range from 7.7 to 16.19 per cent. The mean coefficient of variability for the homozygous-dense group was  $12.00 \pm 0.739$  per cent and ranged from 6.45 to 22.30; the lax group exhibited a mean of  $9.04 \pm 0.636$  per cent and a range from 4.1 to 16.22; the heterozygous group had a mean of  $38.15 \pm 1.818$  per cent with a range from 23.83 to 47.42 per cent for the same character. These figures indicate that the true-breeding F<sub>3</sub> rows are no more variable than the parental rows, but that the heterozygous are about three to four times as variable.

Table 8 proves rather definitely that there were progenies which were homozygous for dense spikes, others which were homozygous for lax spikes, and that the spikes of intermediate length were heterozygous. The rows of homozygous-dense progenies tended to be similar in appearance and density to the F22 parent. The heterozygous group was intermediate between the parents and contained individuals representative of both parental groups, indicating segregation. The homozygous-lax progenies were similar to the Dicklow parent. In only three progenies was the mean density of the Dicklow spike recovered. The mean length of each rachis internode in the F<sub>3</sub> progenies was  $56.5 \pm 1.74$  mm. as compared with  $50.3 \pm 0.577$  mm. for Dicklow. The lax group have a greater length of  $6.2 \pm 1.83$ , which is three and four-tenths times its probable error. The figures indicate a tendency toward transgressive segregation in the direction of a more lax spike than that which characterizes the Dicklow parent. Figure 3 shows the spike density curves.

Table 9 indicates that the range of spike density and the range of coefficients of variability (C. V.) are not far different in homozygous groups of progenies than in either parent. It also proves that the density of the F22 parent was recovered almost identically. The

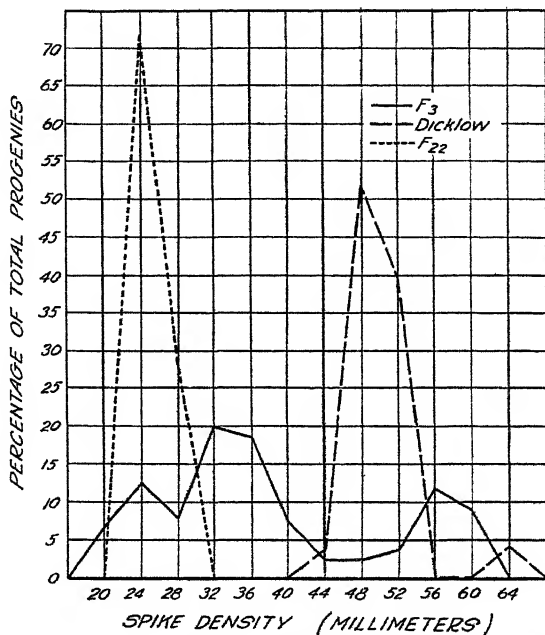


FIGURE 3.—Spike-density curves of the two parents and of the F<sub>3</sub> progenies. One parent, F22, is distinctly more dense than the other parent, Dicklow. In the F<sub>3</sub> progenies there were three definite classes: (1) Homozygous dense with a mean almost identical with F22; (2) homozygous lax which is statistically somewhat more lax than Dicklow; and (3) an intermediate heterozygous group. The three groups overlap somewhat and make a continuous curve but show three peaks, one for each major group. The plants in the homozygous groups were no more variable than the parents



mean spike density of the F22 parent was  $25.6 \pm 0.861$  as compared with a mean of  $25.2 \pm 0.707$  for the homozygous-dense progenies. The heterozygous group could be identified at a glance by the high coefficients of variability. This definitely high coefficient of variability ( $38.15 \pm 1.818$ ) clearly designates the segregating progenies and lends additional evidence to the observation previously made that transgressive segregation probably occurred toward a more lax spike than that of the lax parent, which indicates some sort of additional modifying factor, not clearly isolated by their data. The homozygous-lax progenies, having a mean coefficient of variability of  $10.54 \pm 1.018$  per cent, were easily distinguished from the segregating progenies, with a variability three and six-tenths times as great.

TABLE 9.—Range and mean of mean spike densities for the F22 and Dicklow parent rows, and for three groups of  $F_3$  progenies, together with the range and the mean of the mean coefficients of variability (C. V.) for the same three groups

[Family 35c; grown in 1928 at Logan, Utah]

Strain	Spike-density range	Mean of spike density	C. V. range	Mean of C. V.
	<i>Millimeters</i>	<i>Millimeters</i>	<i>Per cent</i>	<i>Per cent</i>
F22 parent.....	23.8-28.0	25.6	10-18	13.68
Dicklow parent.....	43.5-64.7	50.3	8-16	10.54
Homozygous dense.....	19.5-33.2	25.2	6-22	12.00
Heterozygous.....	24.6-47.6	34.7	24-48	38.15
Homozygous lax.....	49.6-62.4	56.5	4-16	9.04

Stewart (6) found transgressive segregation in both directions in a cross of Kanred  $\times$  Sevier wheat. In the present study, with parent rows placed at 10-row intervals in the nursery, a good comparison was possible. The measurements indicate a rather definite tendency toward greater laxness.

It is evident from the material presented that there are three definite groups of progenies when classified according to spike density. When compared with a 1:2:1 ratio a fair agreement was found. Table 10 compares the closeness of fit on a 1:2:1 segregation basis.  $\chi^2 = 2.6297$  and  $P = 0.2767$ . In 28 cases out of every 100 a worse fit might be expected from chance alone.

TABLE 10.—Closeness of fit of three groups of  $F_3$  progenies on a basis of 1:2:1 segregation for spike density

[Family 35c; grown in 1928 at Logan, Utah]

Group	Observed value (O)	Calculated value (C)	O-C	(O-C) <sup>2</sup>	$\frac{(O-C)^2}{C}$
Homozygous dense.....	60	59.25	0.75	0.5625	0.0095
Heterozygous.....	128	117.50	10.5	110.2500	.9383
Homozygous lax.....	49	59.25	-10.25	105.0625	1.7732

$\chi^2 = 2.7210$ .  $P = 0.2335$ .

#### GLUME COLOR

The F22 parent has a bronze glume of intermediate intensity. The Dicklow parent has a white glume. It has been noted by other workers that bronze is dominant to white. In this family 58 of the

progenies were homozygous for bronze, 110 were heterozygous, and 69 were homozygous for white. Table 11 indicates the number calculated and observed and records  $X^2$  as 2.2405 and  $P=0.3331$ . No color variations outside the range of the parental variations were observed and both parental types were recovered. The fit indicates that in 33 out of 100 cases, a worse fit might be expected, due to chance alone. This fit is satisfactory in indicating that the inheritance is due to a single major factor difference.

TABLE 11.—*Closeness of fit of three groups of  $F_3$  progenies on a basis of 1 : 2 : 1 segregation for glume color*

[Family 35c; grown in 1928 at Logan, Utah]

Group	Observed value (O)	Calculated value (C)	C-O	(C-O) <sup>2</sup>	$\frac{(C-O)^2}{C}$
Homozygous bronze.....	59.25	58	1.25	1.5625	0.0264
Heterozygous.....	118.50	110	8.50	72.25	.6097
Homozygous white.....	59.25	69	-9.75	95.0625	1.6044

$X^2=2.2405$ ,  $P=0.3331$ .

#### CORRELATION STUDIES

In the correlation studies the mean values for the  $F_3$  progenies of each character studied were used.

All possible correlations were made between the four characters for which counts or measurements were taken. The combinations were as follows: (1) Number of culms and culm length; (2) number of culms and awn length; (3) number of culms and spike density; (4) awn length and spike density of all progenies; (5) awn length and culm length; (6) awn length and spike density of homozygous lax and dense progenies; (7) culm length  $F_2$  and  $F_3$ ; and (8) spike density  $F_2$  and  $F_3$ . Table 12 gives a summary of the correlation constants.

TABLE 12.—*Correlation coefficients ( $r$ ), correlation ratios ( $\eta$ ), their respective probable errors (P. E.), and Blakeman's test of linearity for various pairs of plant characters*

[Family 35c; grown in 1928 at Logan, Utah]

Characters correlated	$r \pm P. E.$	$\eta \pm P. E.$	Blakeman's test
Number of culms and culm length.....	$+0.1795 \pm 0.029$	$0.235 \pm 0.013$	1.76
Number of culms and awn length.....	$+ .172 \pm .069$	$.352 \pm .049$	1.886
Number of culms and spike density.....	$+ .167 \pm .0316$	$.425 \pm .028$	5.5
Awn length and spike density (all).....	$+ .142 \pm .035$	$.271 \pm .028$	1.93
Awn length and culm length.....	$- .055 \pm .095$	$.347 \pm .019$	6.05
Awn length (fully awned) and spike density.....	$+ .406 \pm .094$	$.6379 \pm .078$	3.088
Culm length, 1927, and same, 1928.....	$+ .118 \pm .043$	$.164 \pm .0098$	1.306
Spike density $F_2$ and $F_3$ .....	$+ .3785 \pm .0376$	$.809 \pm .048$	10.74
Spike density (homozygous dense and lax) $F_2$ and $F_3$ .....	$+ .383 \pm .085$	.....	.....
Spike density (homozygous dense) and number of culms.....	$- .0796 \pm .033$	.....	.....
Spike density (heterozygous) and number of culms.....	$+ .023 \pm .178$	.....	.....
Spike density (homozygous lax) and number of culms.....	$+ .269 \pm .028$	.....	.....

The correlation coefficient ( $r$ ) and the probable error (P. E.) were calculated for each pair of correlated characters. The correlation ratio  $\eta$  and Blakeman's test for linearity were calculated for the same characters to determine if the regressions were sensibly linear.

When number of culms and culm length were correlated, no significant results were obtained, as  $r$  is only  $0.1795 \pm 0.029$ , rather too small to be very significant. ( $\eta$ ) is  $0.235 \pm 0.013$  and Blakeman's test is only 1.76.

In the correlation between the number of culms and awn length  $r$  is  $0.172 \pm 0.069$  and  $\eta$  is  $0.352 \pm 0.049$ , which is seven times its probable error. Blakeman's test is 1.88. Measurements for awn length were taken only on 47 progenies that were homozygous for awns 4.

The correlation coefficient  $0.167 \pm 0.032$  obtained when number of culms and spike density are correlated is not significant.  $\eta$  approaches significance with a figure of  $0.425 \pm 0.028$ . This figure and Blakeman's test of 5.5 indicates that the correlation is not linear and that  $r$  does not measure all correlation present.

In an effort to determine the cause of this difference found between  $r$  and  $\eta$ , three groups were made of the spike density classes and each group correlated with the number of culms as follows: (1) Homozygous-dense spikes and number of culms; (2) heterozygous spikes and number of culms; and (3) homozygous-lax spikes and number of culms. Homozygous-dense spikes and number of culms, gave a low negative correlation coefficient of  $-0.0796 \pm 0.033$ , which is barely more than twice its probable error. The only correlation in this separation that gave any result approaching significance was where homozygous-lax spike group was correlated with number of culms. The result here was an  $r$  of  $+0.269 \pm 0.028$  which is nine times its probable error but is rather small in absolute value.

In comparing the correlation of awn length and spike density of all progenies neither  $r$  nor  $\eta$  was significant. The same is true of the correlation awn length and culm length.

The only correlation that gave a positive indication of real value was that which measured awn length and spike density for the fully awned group. In this case  $r$  equals  $0.406 \pm 0.094$  and is four and one half times its probable error.  $\eta$  also is large,  $0.6379 \pm 0.078$ . Blakeman's test for linearity, 3.088, indicates that the regression is probably linear. It is concluded, therefore, that there is a definite correlation between the length of the awn and density of spike in the fully awned progenies, that is, as the spike becomes less dense the awn becomes longer (6).

$F_2$  culm length was correlated with  $F_3$  culm length of tall plants. It was found that no correlation existed, as neither  $r$  nor  $\eta$  was large enough to be even slightly important.

Significant correlations were found between the spike density of  $F_2$  plants and that of  $F_3$  progenies, both for all the progenies and for only the homozygous ones. That there is linear correlation is shown by the fact that  $r = +0.378 \pm 0.038$ , about ten times its probable error. The large  $\eta$  of  $0.809 \pm 0.048$  which is seventeen times its probable error and together with a Blakeman's test greater than 10 indicates considerable correlation of a nonlinear nature.

#### SOIL HETEROGENEITY

The Harris method (3) was used to study soil heterogeneity. Single rows of each parent variety were grown side by side after every 10 of the  $F_3$  progeny rows, thus insuring systematic distribution. All the contiguous parental and progeny rows received identical treatment within the range of reasonable error.

Correlation studies were made between culm-length measurements of the F22 and the Dicklow parents and also between the spike-density measurements. The results of these correlations are shown in Table 13.

TABLE 13.—*Correlation studies of parent plant characters, F22 and Dicklow, to determine the presence of soil heterogeneity*

[Family 35c, grown in 1928 at Logan, Utah]

Character studied	Correlation coefficient and probable error
Culm length, Dicklow and F22.....	+0.461±0.111
Spike density, Dicklow and F22.....	+ .626± .085

In the light of the work of Harris showing that variability which produces a positive significant correlation is due to soil heterogeneity, Table 13 proves that heterogeneity of the soil was measurably noticeable in this field. The culm length correlation gave an  $r$  of  $+0.461 \pm 0.111$  and that of spike density an  $r$  of  $+0.626 \pm 0.085$ . Both of these figures are significant in indicating the existence of considerable soil heterogeneity, amounting (when solved by the formula  $v=100(1-\sqrt{1-r^2})$ , where  $V$ =variation in percentage) to about 10 and 20 per cent variability, for culm length and spike density, respectively.

## COMPETITION

The ability of one strain to thrive at the expense of the near-by strain is known as competition. One of the plant characters in this cross influenced by competition is number of culms. A correlation was made between the number of culms produced by each parent when grown side by side after each 10 rows of  $F_3$  progenies. These data gave a correlation ( $r$ ) of  $-0.557 \pm 0.097$ . With respect to number of culms this significant correlation, five and five-tenths times its probable error, indicates a strong competition between F22 and Dicklow. Experimental results with number of culms were influenced by competition to the extent of about 20 per cent of the variability. Such high environmental variability would tend to hide segregation and may perhaps have done so in this study.

## SUMMARY

The cross between F22 (a pure-strain segregate from Dicklow×Sevier) and Dicklow (D3) was made in 1925 at Logan, Utah.

The data were so taken and recorded that the parent and progeny characters could be compared, and in such a manner that correlation studies could be made.

The range of the two parents for culm length was recovered in the progeny, and no segregating groups other than dwarfs were revealed.

The  $F_2$  progenies segregated on a 13 normal:3 dwarf basis for stature. This was plainly indicated when compared with the 7:6:2:1 ratio expected in the  $F_3$ . The Dev./P. E. method was used to separate the classes 13 normal:3 dwarf, the 3:1, and the 1:3 classes. This calculation was successful for the 1:3 class, but only partly so for the 13:3 and the 3:1. The correct numbers exist in other classes and in these two combined. The evidence as a whole bears out the theory that one dominant dwarf factor and an inhibitor factor were operating in the inheritance of stature in this cross.

The F22 and Dicklow parents were essentially alike in number of culms per plant, and no segregation was observed in the progenies.

There was a single-factor difference in awn-class inheritance. The awn types of both parents were recovered. There was also a segregating awn class.

The mean spike density and the coefficient of variability were calculated for each progeny. One major factor difference was found operating in spike-density inheritance. Three groups were recovered: (1) A homozygous-dense group, like the F22 parent; (2) a heterozygous group segregating for spike density; and (3) a homozygous-lax group which tended to be more lax than the Dicklow parent. The means for 10 rachis internodes were  $56.5 \pm 1.74$  mm. for the F<sub>3</sub> homozygous-lax rows and  $50.3 \pm .577$  for the Dicklow parent. The difference is  $6.2 \pm 1.83$ , which is 3.4 times the probable error.

A major and one or more minor factor differences are probably operating to bring about the recovery of the F22 parent spike and the greater laxity of the true-breeding lax progenies as compared with the Dicklow spike. The small coefficient of variability determined for the lax class indicates stability of behavior.

Correlation studies were made of the mean values of each character studies in the F<sub>3</sub> progenies.

The correlation between awn length and spike density in the fully awned progenies gave an  $r$  of  $+0.406 \pm 0.094$ , indicating a correlation between these two characters. Another significant correlation was that which compared spike density in the F<sub>2</sub> progenies with that in the F<sub>3</sub>, indicating stability of behavior for this character.

Soil heterogeneity was studied by using the coefficient of correlation on parent row characters. These paired parental rows were spaced systematically throughout the plot. The correlations were significantly positive for culm length and spike density and indicated that soil heterogeneity was measurably noticeable in this field.

Competition between varieties was studied by correlating the number of culms produced on the two parental rows growing side by side. This gave a high negative correlation, indicating that considerable competition existed between the contiguous rows.

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# AMMONIFICATION OF NITROGENOUS SUBSTANCES BY PURE CULTURES OF MICROORGANISMS<sup>1</sup>

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## INTRODUCTION

Ammonification is strictly a biological process, and a necessary step in obtaining the nitrogen from its organic combination before it can be converted into plant food. Much of the early work done on ammonification and the availability of nitrogen in various nitrogenous materials was conducted in liquid media. Lipman and Burgess (8),<sup>2</sup> however, used a soil medium and compared in detail 15 pure cultures. They found that the accumulation of ammonia was governed by the specific organism, the source of nitrogen, and the type of soil employed.

The results herein reported were obtained with 19 soil organisms in pure cultures acting on a number of nitrogenous substances in a soil medium. The quantity of ammonia produced is used to compare (1) the ease with which the various compounds are ammonified, (2) the inhibitory effect on ammonification of substances not appreciably ammonified, and (3) the relative efficiency of the 19 organisms in causing the accumulation of ammonia in soil.

TABLE 1.—*Summary of morphological and physiological characteristics of micro-organisms<sup>a</sup> used in ammonification studies*

Culture No.	Nitrate reduction	Gelatin liquefaction	Starch hydrolysis	Milk peptonization	Indol production	Litmus-milk reaction	Action on glucose	Action on lactose	Action on sucrose	Shape <sup>b</sup>	Motility	Gram reaction	Chromogenesis	Spore formation
10-B.....	+	+	+	+	+	-	o	o	o	B	+	+	+	+
15-A.....	+	+	+	+	+	-	o	o	o	C	+	+	+	+
101.....	(S)	+	+	+	+	-	o	o	o	B	+	+	+	+
107-A.....	+	+	+	+	+	-	o	o	o	B	+	+	+	+
108-A.....	+	+	+	+	+	-	o	o	o	B	+	+	+	+
112-C.....	+	+	+	+	+	-	o	o	o	B	+	+	+	+
119-A.....	+	+	+	+	+	-	o	o	o	B	+	+	+	+
119-D.....	+	+	+	+	+	-	o	o	o	B	+	+	+	+
119-F.....	+	+	+	+	+	-	+	o	o	B	+	+	+	+
119-G.....	+	+	+	+	+	-	+	o	o	B	+	+	+	+
123-D.....	+	+	+	+	+	o	+	o	+	B	+	+	+	+
125-C.....	+	+	+	+	+	+	+	o	+	B	+	+	+	+
203-A.....	+	+	+	+	+	+	+	+	+	F	+	+	+	+
208-A.....	+	+	+	+	+	+	+	+	+	B	+	+	+	+
210-B.....	+	+	+	+	+	o	+	o	+	B	+	+	+	+
210-D.....	+	+	+	+	+	+	+	o	+	B	+	+	+	+
219-B.....	+	+	+	+	+	+	+	o	+	F	+	+	+	+
221-B.....	+	+	+	+	+	+	+	o	+	B	+	+	+	+
223.....	+	+	+	+	+	+	+	o	+	B	+	+	+	+

<sup>a</sup> All these organisms are aerobic.    <sup>b</sup> B=rods; C=cocci; F=filamentous.    \* Undetermined.

<sup>1</sup> Received for publication Nov. 7, 1930; issued June, 1931. Contribution from the Department of Bacteriology, Utah Agricultural Experiment Station.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 800.

The organisms were isolated from Utah soils by Greaves (5, 6, 7) during the summer of 1928. A summary of their chief physiological and morphological properties as given by him is tabulated in Table 1. Culture 15-B when used in this work was not a pure culture but a mixture of two organisms.

The following compounds were used as sources of nitrogen: Asparagine, dried blood, peptone, gluten, casein, gelatin, egg albumen, urea, uric acid, hippuric acid, acetanilide, diphenylamine, caffeine, and calcium cyanamid.

#### METHOD

Common garden soil, high in calcium carbonate, was dried at 115° C. and so ground as to pass a 20-mesh sieve. Fifty-gram portions were used in common tumblers. Dried blood was added at the rate of 2 per cent. All other compounds were added at the rate of 1 per cent. The soil was thoroughly mixed with the nitrogen carriers, 10 c. c. of distilled water added, and the whole sterilized for two hours at 15 pounds.

Urea and asparagine in solution were added to the soil after separate sterilization, the former by heating for 20 minutes in live steam on three consecutive days and the latter by autoclaving at 15 pounds for 15 minutes. The soils were then inoculated with pure cultures of the organisms grown on nutrient agar. Three checks for each organism and three as controls without inoculation were used in each case. A different source of nitrogen was employed in each set. The same 19 organisms were used in all sets, except when otherwise stated.

After four days' incubation at 27° to 30° C. the ammonia accumulated was determined on the whole sample by distilling and titrating. Magnesium oxide was used as the base. The quantity of ammonia accumulated in the sterile checks in each set was subtracted from that in the inoculated samples to correct for the ammonia formed incident to sterilization and other nonbiological factors. For calculations the average of the three check samples agreeing reasonably well was used.

The ammonia produced in the sterilized soil apart from the added source of nitrogen was small. Seventeen cultures were able to accumulate ammonia varying from 0.2 to 1.5 mgm. This measure was made by inoculating 50 gm. of the sterile soil with pure cultures as above, omitting the introduction of another source of nitrogen.

#### AMMONIFICATION OF ASPARAGINE

Asparagine was readily ammonified by all 19 cultures, but the amount of ammonia accumulated varied over a wide range. Fifty per cent of the organisms showed great activity. Asparagine decomposes in two steps, each yielding one of the two molecules of ammonia it contains. Any organism, therefore, which accumulates more than 50 per cent of the total ammonia brings about, to some extent, both reactions. There were four such organisms. But this need not imply that the activity of those other organisms must be restricted to the first reaction alone. It is quite as logical to suppose that they could cause both reactions but to a less extent, as that they should cause the first step only to occur more completely.

## AMMONIFICATION OF PROTEINACEOUS SUBSTANCES

The more complex compounds, including peptone, casein, gluten, gelatin, dried blood, and egg albumen are acted upon variously by bacteria. Simultaneously with deamination primary splitting of the molecule may be going on and intermediate decomposition products, mostly amines, formed. These changes are brought about by hydrolysis, reduction, and oxidation.

Inasmuch as only 2.5 to 8 per cent (10, p. 144) of the total nitrogen of proteins occurs in the free-NH<sub>2</sub> groups and since as high as 34 per cent of the total nitrogen of gluten, 27 per cent of that of casein, and 11 per cent of that of dried blood were ammonified by pure cultures within four days, it is evident that more than one and probably many reactions are being brought about simultaneously by the same organism.

The results obtained from ammonification of asparagine and the proteinaceous substances, and also from urea, uric acid, and hippuric acid, are tabulated in Table 2 and graphically presented in Figure 1.

Peptone was rather easily ammonified by all 19 organisms. This was to have been expected from the fact that the 19 highest ammonifiers of peptone broth were selected for this work from 68 cultures (5, 6, 7), which also accounts for the comparative uniformity in the ammonifying property of all the organisms on peptone. This ability to split peptone varying from 11 to 42 per cent of the total nitrogen added, points to the common occurrence of the proteolytic enzymes in all cultures.

Seventeen cultures ammonified gluten, the quantity of ammonia ranging from 6 to 33 mgm. or 7 and 38 per cent of the total nitrogen added. Cultures 119-F and 107-A accumulated more ammonia from gluten than from peptone. Cultures 119-A and 221-B did not ammonify gluten.

Casein was ammonified by 18 cultures, the accumulation of ammonia ranging from 1 to 26 mgm., or from 1.4 to 36 per cent of the total added nitrogen. Casein is the only negative protein broken down by ereptase and is used as a substrate to detect its presence (13, p. 107). Therefore erepsin may occur among the caseinases of many of these cultures. It is present in numerous microorganisms (13, p. 213).

All 19 cultures accumulated ammonia from nitrogen supplied in the form of gelatin, the quantity ranging from 1 to 20 mgm. The respective ammonification curves for gelatin and peptone when plotted together suggest a correlation between the ammonification of the two substances, the gelatin being, however, considerably lower than the peptone.

Sixteen cultures ammonified egg albumen; three did not. This again illustrates the specificity of microbial action. Cultures 101 and 10-B were fairly active on the other proteins but did not attack this substrate. Culture 101 was the highest ammonifier of asparagine.

Dried blood was ammonified by all cultures, 18 of which accumulated from 1 to 13 mgm. of ammonia, or between 0.7 and 9 per cent of the total added nitrogen. Culture 203-A accumulated 38 mgm., or nearly three times as much as the highest culture below it. This amount is 26 per cent of the total nitrogen added as shown by analysis. In their experimental work on ammonification of dried blood by pure cultures, Lipman and Burgess (8) conducted experiments in which



they used three different soils as media with 2 per cent dried blood. After 12 days' incubation at 27°-30° C. they found from 6.01 to 24.36 per cent of the nitrogen ammonified by the cultures in sandy soil, from 1.64 to 6.91 per cent in clay-loam soil, and from 2.24 to 9.41

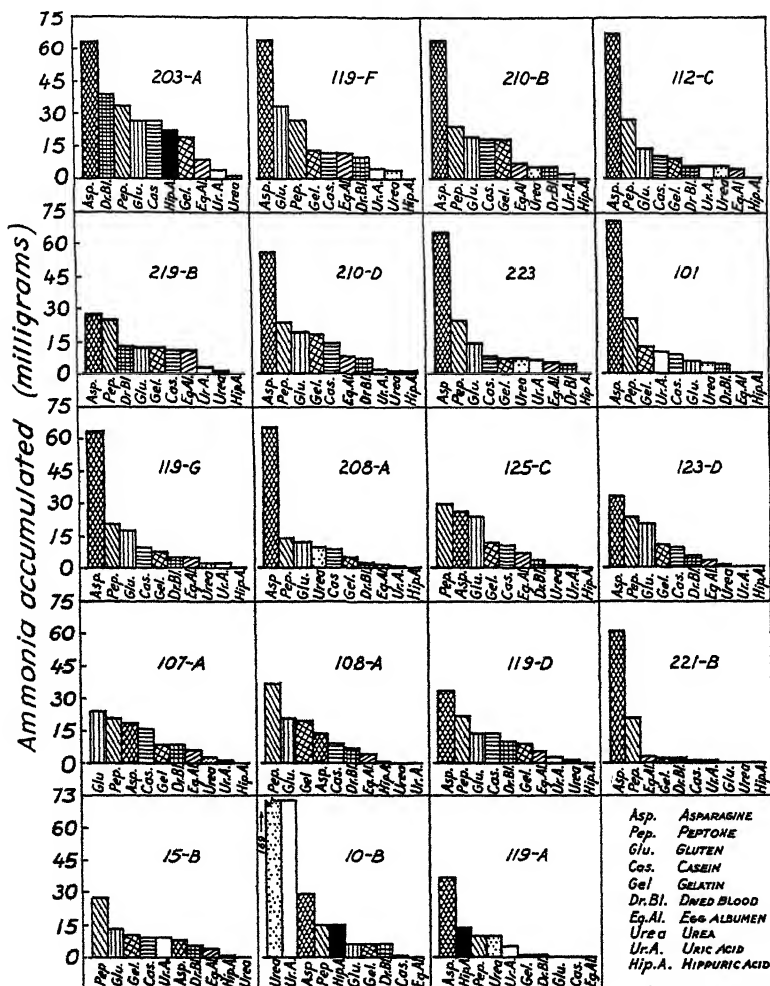


FIGURE 1.—Ammonia accumulated by pure cultures of various organisms in soils containing 10 different sources of nitrogen. The culture number is given in each square

per cent in clay-adobe soil. The decomposition of dried blood is not extremely rapid.

#### AMMONIFICATION OF UREA, URIC ACID, AND HIPPURIC ACID

Sixteen cultures ammonified urea. Culture 10-B was extremely active. Fifty-nine per cent of the nitrogen added was accumulated by this organism, as contrasted with the other cultures, which accumulated not more than 3.5 per cent, represented by 10 mgm. of ammonia.

It has been reported (2) that urea, uric acid, and hippuric acid, in the order named, are increasingly difficult to ammonify. While the total results obtained for the ammonification of urea and uric acid in this work confirm this statement, the margin between the two is very small. In fact, there were more organisms which accumulated more ammonia from uric acid than from urea than the reverse. Table 2 gives the data.

Seventeen cultures ammonified uric acid. Culture 10-B ammonified 36 per cent of the total nitrogen. The other cultures varied from 0.5 to 5 per cent. It is likely that the relationship between the ammonification of uric acid and of urea by culture 10-B is due to the primary formation of urea from uric acid. But, on the other hand, cultures 15-B, 203-A, and 101 represent a group of organisms which accumulate more ammonia from uric acid than from urea.

Hippuric acid, in which the NH is attached only indirectly to the ring was actively ammonified by three cultures and slightly by three others. Culture 203-A accumulated 22 mgm. of ammonia, or 46 per cent of the total nitrogen, and cultures 10-B and 119-A accumulated 15 and 14 mgm., respectively, or about 31 per cent of the total nitrogen.

#### AMMONIFICATION AND CHEMICAL STRUCTURE

Miyake (11) reports that fatty amino compounds ammonify more readily than aromatic amino compounds, that aromatic imino compounds are more difficult to decompose than aromatic amines, and that the nature of the other groups of attachment has no effect on the decomposition of the imino group. Bearing this out, diphenylamine allowed no accumulation of ammonia, while acetanilide having one bond from the NH to the ring was slightly ammonified by five cultures. The ammonia accumulated varied between 0.34 and 1.36 mgm., or 0.6 and 2.2 per cent of the total nitrogen.

TABLE 2.—*Milligrams of ammonia accumulated by pure cultures of various organisms from soil containing 10 different sources of nitrogen*

Culture No.	Milligrams of ammonia accumulated from soil containing—									
	Aspara- gine	Pep- tone	Gluten	Casein	Gelatin	Dried blood	Egg albu- men	Urea	Uric acid	Hip- puric acid
203-A-----	63	33	26	26	19	38	8	1	4	22
119-F-----	64	26	33	12	13	10	12	4	5	-----
210-B-----	64	24	19	18	18	5	7	5	2	-----
112-C-----	67	27	14	10	9	5	4	5	5	-----
219-B-----	28	25	12	11	12	13	11	1	3	-----
210-D-----	56	23	19	14	18	7	8	1	2	1
223-----	65	24	14	8	7	4	5	7	6	-----
101-----	70	25	6	9	12	4	-----	5	10	-----
119-G-----	63	20	17	10	8	5	5	2	2	-----
208-A-----	65	14	12	9	5	3	2	10	1	-----
125-C-----	26	30	24	11	12	4	7	1	1	-----
123-D-----	34	24	21	10	11	6	4	1	1	-----
107-A-----	19	21	24	16	8	8	6	2	1	-----
108-A-----	14	37	21	9	20	7	4	-----	-----	1
119-D-----	34	22	14	14	9	10	5	1	2	-----
221-B-----	61	21	-----	1	2	2	3	-----	-----	-----
15-B-----	8	28	13	9	10	5	4	-----	9	1
10-B-----	29	15	6	2	6	6	-----	169	73	15
119-A-----	37	10	-----	-----	1	1	-----	10	5	14

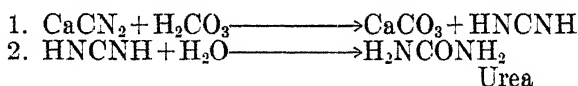
The fact that hippuric acid and other compounds in which the NH group is attached only indirectly to the ring are more easily ammonified than aromatic amines or imines is explained when we know that the initial cleavage is between the ring and the chain attached. Thus with the aid of hystozyme (13, p. 217), an enzyme occurring in fungi and bacteria as well as in animal tissue, hippuric acid is split, forming benzoic acid and glycocoll; the latter is then deaminized. Those bacteria which ammonify hippuric acid also ammonify glycocoll (9, p. 462).

Caffeine in soil inoculated with cultures 203-A, 107-A, and 10-B, respectively, was not ammonified. The structural relationship between caffeine and uric acid can not be taken to indicate a correlation in the ammonification of the two substances.

Nitrogen supplied in the form of a commercial fertilizer, calcium cyanamid, was not measurably ammonified by any of the 19 cultures within the 4-day incubation period. Four different samples of the cyanamid containing by test from 18.76 to 20.38 per cent nitrogen were run separately with the results just indicated. Furthermore, no ammonia was accumulated, above that in the controls, in 50 gm. of the unsterile garden soil to which 0.5 gm. of calcium cyanamid was added.

A series of reactions has been suggested for the changes undergone by calcium cyanamid in the soil forming successively  $\text{Ca}(\text{CNNH})_2$ ,  $\text{CNN}(\text{CaOH})_2$ , and  $\text{CNNCa}_2\text{CO}_3$ . The last-named compound hydrolyzes to give ammonia, calcium carbonate, and calcium hydroxide. Because the dicyanamid is hard to decompose and occurs in dilute concentration within the soil, Löhnis doubts the occurrence of the above reactions (9, p. 590-595).

Calcium cyanamid can undergo a more direct decomposition in the presence of carbonic acid:



Work done by Cowie (4) gives good evidence that calcium cyanamid decomposes in suitable sterile soil, forming urea. Added urease then actively ammonifies this product. The fact that in this work such an active urea ammonifier as culture 10-B failed to accumulate ammonia from cyanamid suggests that the soil used was not suitable to catalyze the reaction by which cyanamid is converted into urea. Cowie's work indicates that this is possible since he found some soils, described as heavy and sandy loams, particularly active catalysts to this reaction, while in other soils no urea was formed.

#### INHIBITORS OF AMMONIFICATION

To test the possible toxicity of acetanilide, diphenylamine, caffeine, and calcium cyanamid, 0.5 gm. of each of these substances was added to 50 gm. of soil containing 1 gm. of dried blood. The soil in the tumblers was inoculated with the six highest ammonifiers of dried blood in pure cultures and incubated four days at 27°-30° C. The data are presented in Table 3.

Diphenylamine totally inhibited a measurable accumulation of ammonia. Furthermore, the quantity of ammonia accumulated by all 19 organisms was greater in sterilized soil than in the presence of diphenylamine.

TABLE 3.—*Inhibiting effect of acetanilide, diphenylamine, caffeine, and calcium cyanamid on ammonification of dried blood (in terms of milligrams of nitrogen accumulated) from soil by various organisms*

Culture No.	Milligrams of ammonia accumulated from soil containing—					Check on sterile soil
	1 gm dried blood	1 gm. dried blood+0.5 gm.—				
		Acetanilide	Diphenylamine	Caffeine	CaCN <sub>2</sub>	
203-A	38.08	0.26		0.34	1.71	
219-B	12.92	1.96		.34	.34	0.85
119-F	10.34	.80		2.21		.85
119-D	9.69	.43		.34		.34
107-A	7.99	.43		.34		1.02
210-D	7.14	.43		1.70		.85

Ammonia was accumulated in small amounts by all six cultures in the presence of acetanilide and of caffeine. This amounted to between 0.01 and 20 per cent of the ammonia accumulated from dried blood without the addition of these. There was a slight ammonification by two cultures and none by a third where calcium cyanamid had been added to the dried blood. It appears from these limited results that these compounds markedly reduce the formation of ammonia, at least during a short period of incubation.

#### AMMONIFICATION IN SOIL v. AMMONIFICATION IN LIQUID MEDIA

In order to compare the accumulation of ammonia in soil and in liquid media, the cultures were inoculated into 1 per cent peptone broth and into 1 per cent urea solution, tap water being used for both. Since the soil and broth sets were incubated separately, and, in the case of urea, analyzed by a different method, the results are comparable only in a general way. The data are plotted in Figure 2.

The ammonification of peptone was twice as great in the soil as in the broth and showed greater variation between cultures. The activity of four cultures in broth approached that in the soil. Culture 119-A was actually higher in the broth. Cultures 203-A, 219-B, 108-A, 15-B, and 119-D showed the greatest contrast, the last-named being six times as efficient, measured in terms of ammonia accumulation, in the soil as in the broth.

In studying the same relationship with urea it is seen that the contrast is just as great but on a smaller scale. The same organisms which showed a small margin between the ammonia accumulated in peptone broth and in the soil do not necessarily show the same small margin between liquid and soil media with urea, and vice versa. Culture 101 accumulated more ammonia from urea solution than from urea in the soil. It was culture 119-A which gave analogous results on peptone, while culture 101 showed wide contrast.

## THE ORGANISMS COMPARED

A comparison of the ammonifying ability of the 19 organisms can best be made by a study of Figure 1. Since an excess of substrate was always present, the ammonification is measured in milligrams of ammonia and not in percentage of the total nitrogen.

Considering the cultures collectively, 203-A was the highest ammonifier on all substances. It occupied first position in ammonifying dried blood, casein, and hippuric acid and was above the average on all other protein substrates but did not appreciably ammonify urea. This culture is a mold which grows luxuriantly on all media. Cultures 119-F, 210-B, and 112-C were active ammonifiers of asparagine and the proteins. While they compare favorably with culture 203-A on asparagine, they were somewhat lower on peptone and casein and

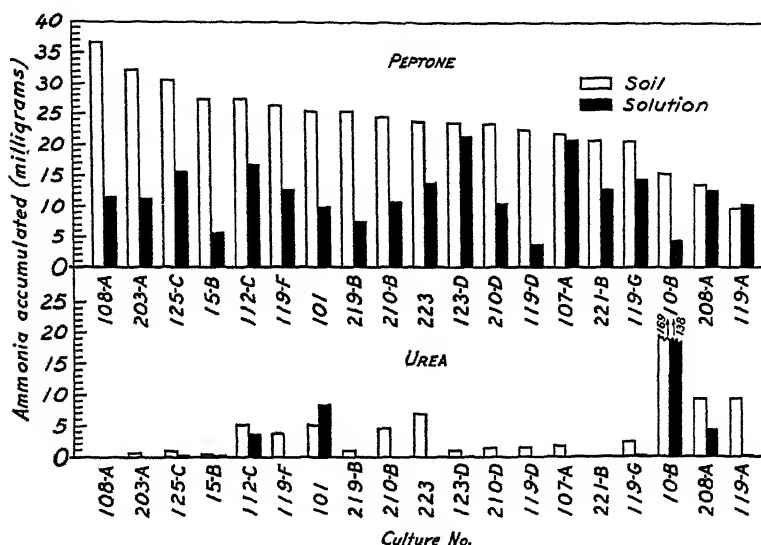


FIGURE 2.—Comparison of results obtained on the ammonification of peptone and urea in soil and in solution by pure cultures of 19 organisms

much lower on dried blood. Culture 119-F was the highest ammonifier of gluten and egg albumen. It ranked second to 203-A in general activity.

Culture 219-B, also a mold, was one of the low ammonifiers of asparagine but was high on dried blood and egg albumen. Its accumulation of ammonia from dried blood, egg albumen, gluten, casein, and gelatin ranged only from 11 to 13 milligrams. The accumulation of ammonia from urea and uric acid by this culture was strictly limited. However, on the whole, its action on all substances was more uniform than that of any other culture.

Culture 101 accumulated no ammonia from egg albumen and little from dried blood, gluten, and casein, but was the highest ammonifier of asparagine. Similarly, culture 221-B showed great activity on asparagine and peptone but very little on all other substrates. But culture 101, unlike 221-B, accumulated 5 milligrams of ammonia from urea and 10 milligrams from uric acid. No. 221-B was inactive on urea and very low on uric acid.

Cultures 107-A, 108-A, and particularly 15-B present an interesting study, for they accumulated more ammonia from peptone, gluten, gelatin (except 107-A), and casein (except 107-A and 108-A) than they did from asparagine. The contrast they present in relation to most of the other organisms is apparent from Figure 1 and from the fact that the average ammonification of asparagine for all cultures was twice that of any other substrate.

Cultures 221-B and 119-A were the lowest ammonifiers of gluten, casein, gelatin, and dried blood. Culture 119-A was also the lowest on peptone and on egg albumen. Interesting enough, it ammonified hippuric acid, uric acid, and urea. Also interesting is the fact that this organism accumulated as much ammonia in peptone broth as in the soil medium, whereas it accumulated none in urea solution despite its activity on urea in the soil.

Culture 10-B is outstanding in its ability to decompose urea and uric acid. Apart from this, it is very comparable to culture 119-A, being low on the proteinaceous materials but also active on hippuric acid. The concentration of ammonia accumulated by this culture in urea media was 0.34 per cent in soil and 0.27 per cent in solution, which is higher than the limit of 0.2 per cent which Bréale (1) claims inhibits ammonification. The question of buffer action of the medium might well be raised, but in the liquid medium such action would be strictly limited, as only the soluble salts present in tap water were added in addition to the urea.

Schellmann (12, p. 22-44) found two out of five urea decomposers and one out of four uric acid decomposers which ammonified hippuric acid. Of the eight highest ammonifiers of urea here reported, two ammonified hippuric acid; of the eight highest ammonifiers of uric acid three ammonified hippuric acid.

Cultures 203-A, 219-B, 119-A, and 119-D are molds. Three of these are relatively low ammonifiers of asparagine. This may be only accidental.

Culture 223 is the median ammonifier on peptone, gluten, and egg albumen and the mode average in five cases. It caused a total accumulation of 154 mgm. of ammonia on all substrates as compared with an arithmetic mean of 160 mgm. for all cultures. Culture 210-D is another "average" ammonifier.

#### SUMMARY

Various compounds used as sources of nitrogen were added to soil and inoculated with pure cultures. The order of availability of the compounds in terms of ammonia accumulated in four days by 19 cultures was asparagine, peptone, gluten, urea, casein, gelatin, dried blood, uric acid, egg albumen, hippuric acid, sterile soil, and acetanilide, with zero results for calcium cyanamid, caffeine and diphenylamine. Urea and uric acid owe their relatively high position to the activity of culture 10-B and would otherwise succeed egg albumen.

Calcium cyanamid does not decompose with the formation of urea in the soil here used. The most active urea organisms and even the unsterile soil did not ammonify the cyanamid.

The ammonifying power of soil microorganisms is quite general on amino nitrogen. The nitrogen groups of the purine bases are resistant to the organisms here studied. There are no known criteria by which the ammonifying property of bacteria can be predicted.

Culture 10-B is an outstanding ammonifier of urea and uric acid. This is quite independent of its relatively low ability to ammonify other combinations of nitrogen.

Ammonification in liquid media is lower than in soil, but there is no definite correlation between the two.

Some nitrogenous substances which are themselves not measurably ammonified retard or prevent the accumulation of ammonia from other sources.

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## EFFECT OF EXTRACTIVES ON THE STRENGTH OF WOOD<sup>1</sup>

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### INTRODUCTION

Studies of the characteristics of wood have brought to light a more or less definite relation between specific gravity and various strength properties.<sup>4,5</sup> The higher the specific gravity, the greater the strength; for comparison the value of specific gravity taken is that for weight oven-dry and volume at test. This relationship, however, manifests itself more as a general trend than as a perfectly uniform law, since observation early established the fact that some individual species depart markedly from average curves for a large number of species. Among the woods exceptional in these relations are many containing relatively large amounts of extractives that add materially to the weight. Some work of the Forest Products Laboratory had already shown that the amount and the character of the extractives may profoundly change other physical characteristics, and it seemed reasonable, therefore, to suppose that the relationship between specific gravity and strength might also be affected. The present study was undertaken to obtain information on the effect of extractives<sup>6</sup> on a limited number of important strength properties.

Redwood (*Sequoia sempervirens*), western red cedar (*Thuja plicata*), and black locust (*Robinia pseudoacacia*) were chosen for the experiments because they contain relatively large amounts of extractives. Redwood particularly has shown rather unusual moisture-strength relations, and when green is high in certain strength properties. It is therefore an especially desirable species for study. In the woods examined the high extractive concentration is confined to the heartwood, the sapwood containing only small amounts. Previous studies at the Forest Products Laboratory on the distribution of extractives in redwood had shown also that the concentration in the heartwood varies considerably with height in tree and with position in cross section of the trunk. The heartwood extractives are highest in the butt adjoining the sapwood and decrease toward the center of the

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<sup>2</sup> Acknowledgement is made to E. C. Sherrard, in charge, section of derived products, Forest Products Laboratory, for assistance in planning and guiding the study; and to both E. C. Sherrard and E. F. Kurth, junior chemist, for all information on the amount of extractives in the material studied.

<sup>3</sup> Maintained by the U. S. Department of Agriculture at Madison, Wis., in cooperation with the University of Wisconsin.

<sup>4</sup> NEWLIN, J. A., and WILSON, T. R. C. THE RELATION OF SHRINKAGE AND STRENGTH PROPERTIES OF WOOD TO ITS SPECIFIC GRAVITY. U. S. Dept. Agr. Bul. 676, 35 p., illus. 1919.

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<sup>6</sup> As here used, extractives are defined as the portion of the wood that will dissolve when the wood is placed in an inert solvent. They are known, for example, as cold-water, hot-water, or alcohol soluble extractives, the name depending upon the solvent used. Total extractive-content values were derived from wood that was finely ground before it was placed in the solvent.



cross section. As the height increases the heartwood extractives gradually decrease near the outside of the tree and increase near the center, so that close to the top the extractive content of the heartwood is about the same throughout the cross section. The extractive content of the sapwood is low and does not vary much throughout the height of the tree.

One inherent difficulty in investigative work like that just mentioned is that the wood put on during successive yearly growth periods may show large variations in strength, so that the best method of selecting or of matching material to eliminate this factor would be to obtain specimens, with and without extractives, from the same annual growth rings. Obviously, this desirability could not be realized, and it was necessary to resort to other methods, which will be described later.

In making comparisons between different groups of material tested, some way of evaluating the significance of extractives in terms of strength is desirable. One method is to adjust the test values for the various strength properties in accordance with the specific gravity-strength equations already determined,<sup>7 8</sup> and to evaluate the effect of extractives in terms of the strength differences that should normally accompany the difference in the specific gravity observed. This method of appraising differences of weight in terms of the theoretical effect of a like amount of wood substance has been employed throughout the study.

## EXPERIMENTAL WORK AND DISCUSSION OF RESULTS

### SCOPE OF TESTS

Four distinct methods were used to determine the effect of extractives on strength, as follows:

(1) Sapwood, which is usually low in extractives, was compared with heartwood, which is relatively high in extractives.

(2) Redwood heartwood from which extractives were partly removed by forcing cold water through in the direction of the grain was compared with adjoining unextracted heartwood.

(3) The outer portion of a kiln-dried redwood block in which the concentration of extractives had been increased by the transfer that is normal during kiln drying was compared with an adjoining interior portion of lower extractive content.

(4) Sapwood of redwood soaked in redwood extractives was compared with normal sapwood.

### COMPARISON OF SAPWOOD AND HEARTWOOD

The sapwood of redwood, western red cedar, and black locust was compared with the heartwood of the same species. Tests<sup>9</sup> were made on material in both the green and the air-dry condition; they included static-bending, compression-parallel-to-grain, and, with redwood, toughness tests also.

<sup>7</sup> See footnote 4 on p. 801.

<sup>8</sup> See footnote 5 on p. 801.

<sup>9</sup> When specimens 2 by 2 inches in cross section were used, the testing procedure followed A. S. T. M. Standard Methods of Testing Small Clear Specimens of Timber (A. S. T. M. Standards, 1927, Pt. II, Nonmetallic Materials, pp. 581-622). Static-bending specimens three-fourths by three-fourths inch in cross section were tested under center loading over a 10-inch span with a testing speed of 0.0304 inch per minute; static-bending specimens five-eighths by five-eighths inch under center loading over an 8-inch span with a testing speed of 0.0288 inch per minute; static-bending specimens one-fourth by one-fourth inch under center loading over a 3½-inch span with a testing speed of 0.0092 inch per minute; compression-parallel-to-grain specimens, in all cases, were four times the least dimension in length, and the testing speed was 0.003 inch per inch of length of specimen; the toughness tests were made on the laboratory toughness-testing machine.

TABLE 1.—Comparison of heartwood and sapwood in the effect of extractives on strength  
(GREEN WOOD)

Compression parallel to grain																
Species	Heart-wood or sap-wood	Trees	Bolt <sup>a</sup>	Annual growth, rings per inch	Hot-water extractives	Specific gravity				Maximum crushing strength						
						Tests	Mois-ture con-tent	Value	Ratio of heart-wood to sap-wood	Heartwood speci-mens—		Value	Ratio of heart-wood to sap-wood		Heartwood speci-mens	
										Above sapwood	Below sapwood		Actual	Calcu-lated <sup>b</sup>	Above sapwood	Below sapwood
		Number	a, e, f, Top	18, 24, 25	Per cent (%)	Number	Per cent	0.374	Per cent	Number	Number	Lbs. per sq. in.	Per cent	Number	Number	
Virgin redwood	H <sup>d</sup>	3	a	18	182.7	58	182.7	0.374	113			4,050	136			
Do.	H <sup>e</sup>	7	e, f	24	117.8	211	117.8	.364				4,210				
Do.	H <sup>f</sup>	6	Top	25	54.0	137	54.0	.336				3,640				
Total		16				406						3,970	117			
Average <sup>f</sup>				23	106.0			.355								
Virgin redwood	S <sup>d</sup>	3	a	31	145.1	9	145.1	.312				2,780				
Do.	S <sup>e</sup>	7	e, f	32	158.1	20	158.1	.325				3,080				
Do.	S <sup>f</sup>	6	Top	34	133.5	20	133.5	.300				2,760				
Total		16				49										
Average <sup>f</sup>				33	146.4			.313				2,910				
AIR-DRY WOOD																
Virgin Redwood	H <sup>d</sup>	3	a	18	11.6	64	11.6	0.405				6,060				
Do.	H <sup>e</sup>	7	e, f	24	11.3	206	11.3	.389				6,140				
Do.	H <sup>f</sup>	6	Top	25	11.2	142	11.2	.368				5,680				
Total		16				412		.360	109				117	111		
Average <sup>f</sup>				23			11.3	.350				5,960				
Virgin redwood	S <sup>d</sup>	3	a	32	11.4	6	11.4	.355				4,970				
Do.	S <sup>e</sup>	7	e, f	31	11.0	23	11.0	.357				5,290				
Do.	S <sup>f</sup>	6	Top	34	11.6	20	11.6	.340				4,880				
Total		16				49										
Average <sup>f</sup>				32			11.3	.350				5,080				

Footnotes at end of table.

TABLE 1.—Comparison of heartwood and sapwood in the effect of extractions on strength—Continued  
GREEN VIRGIN REDWOOD

Heartwood or sapwood	Static bending										
	Specific gravity					Modulus of rupture			Work to maximum load		
	Tests	Mois- ture con- tent	Value	Ratio of heart- wood to sap- wood	Heartwood speci- mens—		Value	Ratio of heart- wood to sap- wood		Heartwood speci- mens—	
					Above sapwood	Below sapwood		Actual	Calcu- lated <sup>b</sup>		Actual
Heartwood <sup>a</sup>	Number	Per cent		Per cent	Number	Number	Lbs. per sq. in.	Per cent	Per cent	Number	Number
Do.	30	178.2	0.360				6,940				
Do.	98	.376					7,480				
Do.	64	.354					6,570				
Total	192										
Average <i>f</i>		108.1	.355	111			7,040	119	117	105	123
Sapwood <sup>a</sup>	3	167.3	.337				5,900				
Do.	10	.334 <td></td> <td></td> <td></td> <td></td> <td>6,100</td> <td></td> <td></td> <td></td> <td></td>					6,100				
Do.	8	.316					5,650				
Total	21										
Average <i>f</i>		134.5	.328				5,890				
AIR-DRY VIRGIN REDWOOD											
Heartwood <sup>a</sup>	27	11.7	0.398				8,900				
Do.	94 <td>.394<td></td><td></td><td></td><td><td>9,820<td></td><td></td><td></td><td></td></td></td></td>	.394 <td></td> <td></td> <td></td> <td><td>9,820<td></td><td></td><td></td><td></td></td></td>				<td>9,820<td></td><td></td><td></td><td></td></td>	9,820 <td></td> <td></td> <td></td> <td></td>				
Do.	54 <td>.360<td></td><td></td><td></td><td><td>8,710<td></td><td></td><td></td><td></td></td></td></td>	.360 <td></td> <td></td> <td></td> <td><td>8,710<td></td><td></td><td></td><td></td></td></td>				<td>8,710<td></td><td></td><td></td><td></td></td>	8,710 <td></td> <td></td> <td></td> <td></td>				
Total	175										
Average <i>f</i>		11.5	.382	109			9,230	105	114	79	119
Sapwood <sup>a</sup>	4	11.6	.350				8,370				
Do.	10 <td>.368<td></td><td></td><td></td><td><td>9,550</td><th></th><th></th><th></th><th></th></td></td>	.368 <td></td> <td></td> <td></td> <td><td>9,550</td><th></th><th></th><th></th><th></th></td>				<td>9,550</td> <th></th> <th></th> <th></th> <th></th>	9,550				
Do.	11 <td>.334<td></td><td></td><td></td><td><td>8,260<th></th><th></th><th></th><th></th></td></td></td>	.334 <td></td> <td></td> <td></td> <td><td>8,260<th></th><th></th><th></th><th></th></td></td>				<td>8,260<th></th><th></th><th></th><th></th></td>	8,260 <th></th> <th></th> <th></th> <th></th>				
Total	25										
Average <i>f</i>		11.8	.352				8,840 <th></th> <th></th> <th></th> <th></th>				

**GREEN VIRGIN REDWOOD**

Toughness <sup>c</sup>										
Heartwood or sapwood	Mois- ture content	Specific gravity		Radial toughness			Tangential toughness			
		Value	Ratio of hardwood to sap- wood	Tests	Value	Ratio of heart- wood to sapwood		Tests	Value	Ratio of heart- wood to sapwood
						Actual	Calcu- lated <sup>b</sup>			
Heartwood <sup>a</sup>	Per cent		Per cent	Number	In.-lbs per specimen	Per cent	Per cent	Number	In.-lbs. per specimen	Per cent
Do.	180.7	0.390		20	67.9			21	98.3	
Do.	115.3	.389		57	55.6			68	92.3	
Do.	51.3	.362		34	43.3			33	60.2	
Total				111				112		
Average <sup>d</sup>	103.6	.376	110		53.3	83	121		81.6	78
Sapwood <sup>a</sup>	Per cent									
Do.	185.8	.338		3	68.4			1	92.9	
Do.	135.4	.336		8	71.6			3	87.3	
Do.	120.3	.350		5	52.8			4	131.4	
Total				16				8		
Average <sup>d</sup>	139.2	.341			64.0				104.9	

AIR-DRY VIRGIN REDWOOD

[illegible]

Footnotes at end of table.



## GREEN WOOD

Black locust.....	H <sup>d</sup> H	1 1	a a	6½ 7½	9.36 9.84	6 6	27.6 38.0	0.659 .646			6 6	6,190 5,820		6 6
Do.....														
Total Average f.....		2		7	9.60	12	32.8	.653	110		12	6,000	148	113
Black locust.....	S <sup>d</sup> S	1 1	a a	10 11	6.44 6.15	6 6	32.7 37.3	.605 .580				4,120 3,960		
Do.....														
Total Average f.....		2		10½	6.30	12	35.0	.592				4,060		

## AIR-DRY WOOD

Black locust.....	H <sup>d</sup> H	1 1	a a	7½ 7	9.98 9.79	6 6	8 8	0.703 .652			5 6	12,240 11,500		6 6
Do.....														
Total Average f.....		2		7	9.89	12	8	.678	110		11	11,870	140	113
Black locust.....	S <sup>d</sup> S	1 1	a a	13 12	6.30 5.95	6 6	8 8	.620 .609				8,720 8,230		
Do.....														
Total Average f.....		2		12½	6.13	12	8	.615				8,480		

Footnotes at end of table.

TABLE 1.—Comparison of heartwood and sapwood in the effect of extractions on strength—Continued  
GREEN WESTERN RED CEDAR

Heartwood or sapwood	Static bending															
	Tests	Mois- ture con- tent	Specific gravity				Modulus of rupture				Work to maximum load					
			Ratio of heart- wood to sap- wood		Heartwood speci- mens—		Value	Ratio of heart- wood to sap- wood		Heartwood speci- mens—		Value	Ratio of heart- wood to sap- wood		Heartwood speci- mens—	
			Above sapwood	Below sapwood	Number	Per cent		Actual	Calcu- lated <sup>b</sup>	Number	Per cent		Actual	Calcu- lated <sup>b</sup>	Number	Per cent
Heartwood <i>d</i> .....	Number	Per cent			Number	Per cent	Lbs. per sq. in.	Per cent	Number	Per cent	In lbs. per cu. in.	Per cent	Number	Per cent	In lbs. per cu. in.	Per cent
Do.....	12	36.6	0.313	113	11	1	5,460	124	12	0	7.13	111	6	6	6	6
Do.....	9	38.2	.321	.....	9	0	5,770	.....	9	0	6.40	.....	7	7	7	7
Do.....	13	31.3	.325	.....	13	0	6,410	.....	13	0	8.72	.....	0	0	0	0
Total.....	34	.....	.....	.....	33	1	.....	.....	34	0	.....	.....	19	19	19	19
Average <i>f</i> .....	.....	35.4	.320	113	.....	.....	5,880	124	120	.....	7.13	111	128	.....	.....	.....
Sapwood <i>d</i> .....	12	75.2	.263	.....	.....	.....	4,320	.....	.....	.....	5.97	.....	.....	.....	.....	.....
Do.....	9	86.0	.283	.....	.....	.....	4,760	.....	.....	.....	5.42	.....	.....	.....	.....	.....
Do.....	13	182.0	.304	.....	.....	.....	5,160	.....	.....	.....	7.83	.....	.....	.....	.....	.....
Total.....	34	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average <i>f</i> .....	.....	97.7	.283	.....	.....	.....	4,750	.....	.....	.....	6.41	.....	.....	.....	.....	.....
AIR-DRY WESTERN RED CEDAR																
Heartwood <i>d</i> .....	12	10	0.333	.....	12	0	9,430	.....	.....	12	0	8.88	.....	.....	7	7
Do.....	8	10	.341	.....	8	0	10,250	.....	.....	8	0	9.60	.....	.....	3	3
Do.....	12	10	.343	.....	11	1	10,280	.....	.....	11	1	11.70	.....	.....	6	6
Total.....	32	10	.....	112	31	1	.....	.....	119	31	1	.....	.....	.....	16	16
Average <i>f</i> .....	.....	.....	.339	.....	.....	.....	9,900	119	118	.....	.....	10.10	105	125	.....	.....
Sapwood <i>d</i> .....	12	10	.287	.....	.....	.....	7,650	.....	.....	.....	.....	8.07	.....	.....	.....	.....
Do.....	8	10	.303	.....	.....	.....	8,410	.....	.....	.....	.....	8.98	.....	.....	.....	.....
Do.....	12	10	.323	.....	.....	.....	9,200	.....	.....	.....	.....	11.70	.....	.....	.....	.....
Total.....	32	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average <i>f</i> .....	.....	10	.304	.....	.....	.....	8,420	.....	.....	.....	.....	9.58	.....	.....	.....	.....

## GREEN BLACK LOCUST

Heartwood <i>d</i> .....	6	45.9	0.680	.....	6	0	14,460	.....	6	0	32.8	.....	4
Do.....	6	58.1	.637	.....	6	0	14,030	.....	6	0	30.7	.....	5
Total.....	12	52.0	.658	113	12	0	14,240	133	120	0	31.8	116	128
Average <i>f</i> .....													9
Sapwood <i>d</i> .....	6	44.4	.596	.....	6	0	10,940	.....	6	0	28.4	.....	3
Do.....	6	49.8	.571	.....	6	0	10,450	.....	6	0	26.3	.....	3
Total.....	12	47.1	.584	.....	12	0	10,700	.....	12	0	27.4	.....	3
Average <i>f</i> .....													

## AIR-DRY BLACK LOCUST

Heartwood <i>d</i> .....	6	8	0.728	.....	6	0	27,100	.....	6	0	41.1	.....	4
Do.....	6	8	.700	.....	6	0	26,100	.....	6	0	36.3	.....	4
Total.....	12	8	.714	109	12	0	26,600	134	114	0	38.7	119	8
Average <i>f</i> .....													4
Sapwood <i>d</i> .....	6	8	.664	.....	6	0	20,700	.....	6	0	36.3	.....	4
Do.....	6	8	.649	.....	6	0	19,050	.....	6	0	30.7	.....	4
Total.....	12	8	.656	.....	12	0	19,880	.....	12	0	33.5	.....	4
Average <i>f</i> .....													

<sup>a</sup> The letter "a" designates the first log section or bolt, 4 feet in length, taken above the stump; "b" the second 4-foot bolt; and so forth.

<sup>b</sup> Calculated heartwood value =  $\left\{ \frac{\text{Heartwood sp. gr.}}{\text{Sapwood sp. gr.}} \right\}^x$  (sapwood strength value). The values of *x* are: For maximum crushing strength, 1.25; modulus of rupture, 1.50; work to maximum load, 2.00; and toughness, 2.00.

<sup>c</sup> Specimens  $\frac{5}{8}$  by  $\frac{3}{8}$  by 10 inches were tested over an 8-inch span with center loading.

<sup>d</sup> The first entry in each comparable group reports specimens from the same trees, and the succeeding entries in the same groups correspond similarly.

<sup>e</sup> A large number of determinations made in an earlier study of similar material indicate that the hot-water extractives of virgin redwood heartwood average about 10 to 15 per cent.

<sup>f</sup> The individual values were weighted in accordance with the number of trees represented.



## REDWOOD

The redwood static-bending and compression-parallel-to-grain specimens were 2 by 2 inches in cross section. Adjoining specimens, cut from a strip extending across the full section as indicated in Figure 1, A, were used. The toughness specimens, which were  $\frac{5}{8}$  by  $\frac{5}{8}$  by 10 inches, were cut from the uninjured end of the static-bending specimens after the first test. The results of all these tests are given in Table 1.

The averages of the results of tests of both heartwood and sapwood specimens from the butt (bolt a),<sup>10</sup> second log (bolts e-f), and top of merchantable length are given. The results are also expressed as ratios of heartwood to sapwood. In addition, calculated ratios of heartwood to sapwood are presented; these are the ratios to be expected if extractives increase the strength of wood in the same ratio as an equal weight of wood substance. The exact method of calculation is given in footnote b of Table 1.

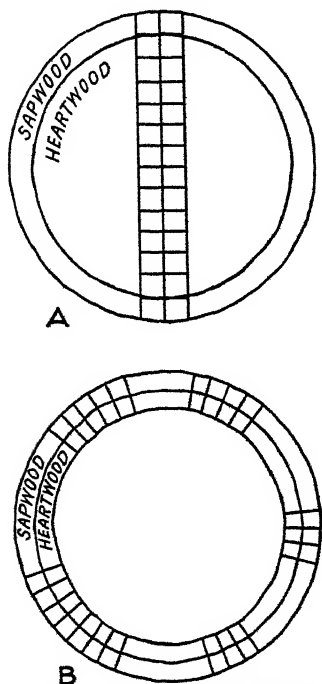


FIGURE 1.—Diagram for the cutting of samples: A, virgin redwood; B, western red cedar and black locust

The specific gravity of the heartwood of redwood, as shown by tests on green material, is approximately 10 per cent higher than that of the sapwood, a fact that indicates higher strength values for heartwood than for sapwood. Actual ratios compared with calculated ones showed that the maximum crushing of the strength of the green heartwood was higher than that of the sapwood by an amount about twice as great as that which would be expected from a like increase of wood substance. Modulus of rupture showed about the difference indicated by the calculated ratios, while in work to maximum load the increase was considerably less, the heartwood being only slightly higher than the sapwood in this property. The toughness tests showed the heartwood to be actually lower than the sapwood, although the difference in weights indicates considerably higher values. The increase in the weight of redwood through the infiltration of extractives, and perhaps other substances, apparently does not affect all properties of green wood in the same way; the change increases some properties, has little effect on others, and actually reduces still others. When a property, work to maximum load for instance, is in a general way inversely proportional to another property, compressive strength in this instance, an increase in the compressive strength will usually decrease the work to maximum load.

When redwood was tested in the air-dry condition, the difference in strength between heartwood and sapwood was not the same as for a

<sup>10</sup> The letter "a" designates the first log section or bolt, 4 feet in length, taken above the stump, "b" the second 4-foot bolt, and so forth.

green condition. The tests showed that the compressive strength of the heartwood as compared with that of the sapwood was higher than the value that would result from a like difference in weight of wood substance, but not to the degree of the redwood tested in a green condition; similarly the modulus of rupture of air-dry heartwood was proportionally somewhat lower, and the work to maximum load much lower, in fact even lower than the lighter weight sapwood. The ratios from the toughness tests were similar to those from work-to-maximum-load values; both properties are measures of shock resistance.

The tests of both green and air-dry redwood indicate that redwood heartwood as compared to sapwood on a weight-strength basis is considerably higher in maximum crushing strength in compression parallel to grain, slightly higher in modulus of rupture, and in most instances lower in shock resistance. Since, as far as is known, there are no structural differences between heartwood and sapwood that could account for the differences in strength, and since, as is shown later, the infiltration and removal of extractives affect the strength, probably these differences in strength are primarily due to extractives.

#### WESTERN RED CEDAR

Sapwood and adjoining heartwood specimens were so selected as to be representative of the entire outer cross section of the tree, as indicated in Figure 1, B. Only trees with sapwood and adjoining heartwood of approximately the same rate of growth were used. Compression-parallel-to-grain and static-bending tests were made. The sapwood in western red cedar is relatively thin and so, to obtain specimens that were all sapwood, relatively small pieces three-fourths by three-fourths inch in cross section were tested.

The results of tests of green western red cedar as shown in Table 1 are similar to those of green redwood; the maximum crushing strength of the heartwood exceeds that of the sapwood by an amount greater than that which the calculated ratios indicate and the actual ratios of moduli of rupture are about equal to the calculated ratios, while the work to maximum load of the heartwood does not exceed that of the sapwood to the extent calculated from the weight-strength ratios.

Extractives apparently affect the strength of air-dry western red cedar in about the same manner as when it is green, while with redwood the effect was somewhat less.

#### BLACK LOCUST

The test specimens of black locust were selected in the same manner as those of western red cedar. (Fig. 1, B.) In selecting trees for test purposes, uniform rate of growth of sapwood and adjoining heartwood was the principal factor considered; even so, a considerable range in rate of growth existed. The sapwood in black locust is extremely thin and, to obtain specimens entirely of sapwood, pieces only one-fourth by one-fourth inch in cross section were used. Compression-parallel-to-grain and static-bending tests with the wood in both a green and an air-dry condition were also made on this species.

The increase in the strength of black locust heartwood as compared with the sapwood was more than for either redwood or western red

cedar. (Table 1.) The maximum crushing strength in compression-parallel-to-grain and also the modulus of rupture of the heartwood as compared with those of the sapwood are considerably higher than the values that would result from like differences in the amount of wood substance. For green wood, the increase in work to maximum load can be accounted for without recourse to extractives, but for air-dry wood the extractives must be included as a cause of this increase. Although the difference between heartwood and sapwood in work to maximum load is not so large as the difference in weight would indicate, still it is much more in line with the weight-strength ratios than for redwood and western red cedar.

Throughout this article, as already suggested, the evaluations of test results are made as if the additional weight of extractives in a piece of wood were an additional weight of wood substance. Further, even though the extractives may have actually increased the strength, if the increase was less than that which an equal weight of wood substance would have given, then the percentages in the tables indicate in general a failure to reach full expected increase rather than a minor increase. Black locust, however, differs from redwood and western red cedar enough to make desirable an additional analysis of part of the results for it. Unlike the other species, the sapwood of black locust as well as the heartwood is relatively high in extractives, and the difference in weight between the heartwood and the sapwood tested is therefore due primarily to wood substance rather than to extractives. In the calculations made during the analysis, but not reported here, the specific-gravity values for this species were corrected to those for wood substance alone; the results showed that the increase in wood substance itself is sufficient to account for the increase in work to maximum load of green black locust heartwood as compared with the sapwood. On the other hand, the extractives must be considered a contributing cause for such increase in air-dry black locust.

#### EFFECT OF REMOVING EXTRACTIVES

If the addition of extractives in the natural physiological change of sapwood into heartwood increases some of the strength properties of certain woods, the removal of these extractives should be expected to decrease these strength properties. In order to determine the effect on the strength of removing extractives, three series of tests comparing extracted to unextracted wood were made, as follows:

*Series 1.*—Compression-parallel-to-grain tests of specimens cut from redwood blocks 6 inches in length.

*Series 2.*—Compression-parallel-to-grain and static-bending tests of specimens cut from redwood blocks 24 inches in length.

*Series 3.*—Compression-parallel-to-grain and static-bending tests of specimens cut from white spruce blocks 11 inches or 18 inches in length.

The tests on white spruce (series 3), which is low in extractives, were included to determine whether any change in mechanical properties was due to method rather than to the removal of the extractives themselves.

TABLE 2.—Average strength values of wood from which extractives were partly removed, by forcing cold water along the grain, compared with the values of unextracted wood

## GREEN WOOD

Species	Series No.	Kind of specimen	Tree No.	Length of piece	Cross section of specimen	Time of extraction	Hot-water extractives	Moisture content	Specific gravity				Maximum crushing strength			
									Value	Ratio of value to base <sup>a</sup>	Specimens --		Value	Ratio of value to base <sup>a</sup>	Specimens --	
											Above base <sup>a</sup>	Below base <sup>a</sup>			Above base <sup>a</sup>	Below base <sup>a</sup>
							No.	Per cent		Per cent	No.	No.		Per cent	No.	No.
Virgin redwood	1	Extracted wood	65	Ins.	$\frac{1}{2} \times \frac{1}{2}$	Days	Per cent	Per cent								
Do.	1	do.	66	6	$\frac{1}{2} \times \frac{1}{2}$	69	6.11	102.2	.388	91	0	8		8	0	8
						78	4.08	103.9	.383	94	1	8		8	0	9
Total						74	5.10	104.0	.386	92	1	10		83	0	17
Average																91
Virgin redwood	1	Adjacent control <sup>b</sup>	65	6	$\frac{1}{2} \times \frac{1}{2}$		13.52	50.1	.428							
Do.	1	do.	66	6	$\frac{1}{2} \times \frac{1}{2}$		8.95	46.2	.400							
Total							34	46.2	.417							
Average							11.28									

## AIR-DRY WOOD

Species	Series No.	Kind of specimen	Tree No.	Length of piece	Cross section of specimen	Time of extraction	Hot-water extractives	Moisture content	Specific gravity				Maximum crushing strength			
									Value	Ratio of value to base <sup>a</sup>	Specimens --		Value	Ratio of value to base <sup>a</sup>	Specimens --	
											Above base <sup>a</sup>	Below base <sup>a</sup>			Above base <sup>a</sup>	Below base <sup>a</sup>
							No.	Per cent		Per cent	No.	No.		Per cent	No.	No.
Virgin redwood	1	Extracted wood	65	6	$\frac{1}{2} \times \frac{1}{2}$	69	6.11	9.4	0.400	92	0	8		8	0	8
Do.	1	do.	66	6	$\frac{1}{2} \times \frac{1}{2}$	78	4.08	10.1	.389	96	1	8		80	1	8
Total						74	5.10	9.8	.403	94	1	10		80	1	16
Average																93
Virgin redwood	1	Adjacent control <sup>b</sup>	65	6	$\frac{1}{2} \times \frac{1}{2}$		13.62	9.4	.442							
Do.	1	do.	66	6	$\frac{1}{2} \times \frac{1}{2}$		8.95	10.1	.416							
Total							34									
Average							11.28	9.8	.426							

Footnotes at end of table.

TABLE 2.—Average strength values of wood from which extractions were partly removed, by forcing cold water along the grain, compared with the values of unextracted wood—Continued

## GREEN WOOD

Species	Series No.	Kind of specimen	Tree No.	Length of piece	Cross section of specimen	Time of extraction	Hot-water extractions	Compression parallel to grain										
								Tests	Specific gravity			Maximum crushing strength						
									Moisture content	Ratio of value to base <sup>a</sup>		Specimens—Above base <sup>a</sup>	Specimens—Below base <sup>a</sup>	Value	Ratio of value to base <sup>a</sup>	Specimens—		Specific gravity ratio to 1.25 power
										Above base <sup>a</sup>	Below base <sup>a</sup>					Above base <sup>a</sup>	Below base <sup>a</sup>	
							No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent		
Virgin redwood	2	Extracted wood	65	24	$\frac{3}{4} \times \frac{3}{4}$	124	5.89	70.0	.410	0	8	4,310	88	1	8			
Do.	2	do.	66	24	$\frac{3}{4} \times \frac{3}{4}$	120	4.08	116.6	.368	2	12	3,360	86	0	14			
Total Average						122	4.98	93.3	.389	2	20	3,840	87	1	22	95	4,180	
Virgin redwood	2	Adjacent control <sup>b</sup>	65	24	$\frac{3}{4} \times \frac{3}{4}$		10.65	60.8	.433			4,890						
Do.	2	do.	66	24	$\frac{3}{4} \times \frac{3}{4}$		8.37	88.9	.378			3,910						
Total Average							9.53	74.8	.406			4,400						
Virgin redwood	2	Separate control <sup>c</sup>	65		$\frac{3}{4} \times \frac{3}{4}$		13.30	65.3	.442	4	1	4,960	101	2	3			
Do.	2	do.	66		$\frac{3}{4} \times \frac{3}{4}$		10.32	81.2	.397	7	0	4,220	108	7	0			
Total Average							11.81	73.2	.420	11	1	4,590	104	9	3	105	4,620	

## AIR-DRY WOOD

Virgin redwood.....	2	Extracted wood.....	65	24	$\frac{3}{4} \times \frac{1}{4}$	124	5.89	10	13.0	0.422	97	4	6	99	6,600	4	6	99	6,600
Do.....	2	do.....	66	24	$\frac{3}{4} \times \frac{1}{4}$	120	4.08	14	11.3	.380	97	2	7	96	5,790	7	7	96	5,790
Total.....						122	4.98	24	12.2	.400	97	6	13	95	6,200	11	13	95	6,100
Average.....																			
Virgin redwood.....	2	Adjacent control <sup>b</sup> .....	65	24	$\frac{3}{4} \times \frac{1}{4}$		10.68	10	13.0	.433					6,680				
Do.....	2	do.....	66	24	$\frac{3}{4} \times \frac{1}{4}$		8.37	14	11.3	.402					6,000				
Total.....								24	12.2	.418					6,340				
Average.....							9.52												
Virgin redwood.....	2	Separate control <sup>c</sup> .....	65		$\frac{3}{4} \times \frac{1}{4}$		13.30	5	13.0	.458	106	5	0	100	7,250	5	0		
Do.....	2	do.....	66		$\frac{3}{4} \times \frac{1}{4}$		10.52	7	11.3	.406	101	4	3	99	5,940	3	4		
Total.....								12	12.2	.452	104	9	3		6,600	8	4		
Average.....							11.81												

Footnotes at end of table.



## AIR-DRY VIRGIN REDWOOD

Extracted wood	2	5	13.0	0.425	96	1	4	11,700	100	3	2	---	---	10.85	110	4	1	---
Do	2	7	11.3	.387	98	1	6	9,190	93	3	4	---	---	7.98	97	3	4	---
Total		12	12.2	.406	97	2	10	10,440	96	6	6	---	---	9.42	104	7	5	---
Average												96	10,350					8.51
Adjacent control	2	5	13.0	.442	---	---	---	11,700	---	---	---	---	---	9.90	---	---	---	---
Do	2	7	11.3	.394	---	---	---	9,880	---	---	---	---	---	8.20	---	---	---	---
Total		12	12.2	.418	---	---	---	10,790	---	---	---	---	---	9.05	---	---	---	---
Average					---	---	---											
Separate control	2	5	13.0	.460	104	5	0	11,800	101	2	3	---	---	9.18	93	3	2	---
Do	2	7	11.3	.407	103	4	3	10,620	108	5	2	---	---	8.59	105	4	3	---
Total		12	12.2	.434	104	9	3	11,210	104	7	5	---	---	8.88	99	7	5	---
Average												106	11,430					9.77

Footnotes at end of table.



TABLE 2.—Average strength values of wood from which extractives were partly removed, by forcing cold water along the grain, compared with the values of unextracted wood—Continued

## GREEN WOOD

Species	Series No.	Kind of specimen	Tree No.	Length of piece	Cross section of specimen	Time of extraction	Hot-water extractions	Compression parallel to grain				Maximum crushing strength			
								Specific gravity		Specimens—		Ratio of value to base <sup>a</sup>	Specimens—		Specific gravity ratio to 1.25 power
								Moisture content	Value	Ratio of value to base <sup>a</sup>	Above base <sup>a</sup>	Below base <sup>a</sup>	Value	Ratio of value to base <sup>a</sup>	Base multiplied by value
								Tests			No.	No.			
White Spruce	3	Extracted wood	196	11	1 1/2 x 1 1/2	20	Per cent	No.	Per cent	Per cent	No.	No.	Lbs. per sq. in.	Per cent	Lbs. per sq. in.
	3	do	196	18	5/8 x 5/8	93	2.04	8	83.1	0.369	7	5	2,410	102	2,450
		Total Average				61		15	109.2	.355	12	3	2,340	100	
White spruce	3	Adjacent control <sup>b</sup>	196	11	1 1/2 x 1 1/2		2.60	16	51.1	.350			2,360		
	3	do	196	18	5/8 x 5/8			7	73.4	.335			2,300		
		Total Average						23	62.3	.343			2,330		
White spruce	3	Separate control <sup>c</sup>	196		1 1/2 x 1 1/2		2.53	14	36.3	.348	4	10	2,290	98	
	3	do	196		5/8 x 5/8			7	31.6	.349	6	5	2,400	108	
		Total Average						21	34.0	.348	10	11	2,410	103	2,380

## AIR-DRY WOOD

White spruce.....	3	Extracted wood.....	196	11	$\frac{1}{2} \times \frac{1}{2}$	29	8	11.2	0.386	100	5	3	5,830	102	7	1	---
Do.....	3	do.....	196	18	$\frac{1}{2} \times \frac{1}{2}$	93	7	13.0	.366	102	5	2	5,230	108	6	1	---
Total.....						61	15	12.1	.376	101	10	5	5,530	105	13	2	---
Average.....																	5,340
White spruce.....	3	Adjacent control b.....	196	11	$\frac{1}{2} \times \frac{1}{2}$		16	11.1	.384				5,710				---
Do.....	3	do.....	196	18	$\frac{1}{2} \times \frac{1}{2}$		7	13.0	.357				4,850				---
Total.....							23	12.0	.370				5,280				---
Average.....																	---
White spruce.....	3	Separate control c.....	196		$\frac{1}{2} \times \frac{1}{2}$		14	11.0	.388	101	9	5	6,020	106	11	3	---
Do.....	3	do.....	196		$\frac{1}{2} \times \frac{1}{2}$			13.0	.373	104	6	1	4,620	95	3	4	---
Total.....							21	12.0	.380	102	15	6	5,320	100	14	7	---
Average.....																	5,440

Footnotes at end of table.

TABLE 2.—Average strength values of wood from which extractives were partly removed, by forcing cold water along the grain, compared with the values of unextracted wood—Continued

## GREEN WHITE SPRUCE

 $\frac{5}{8}$  x  $\frac{5}{8}$  inch in cross section

Kind of specimens	Series No.	Static bending											
		Specific gravity			Modulus of rupture			Work to maximum load			Specific gravity ratio to 2 00 power		
		Moisture content	Ratio of value to base <sup>a</sup>		Value	Ratio of value to base <sup>a</sup>		Value	Ratio of value to base <sup>a</sup>		Value	Ratio of value to base <sup>a</sup>	
			Above base <sup>a</sup>	Below base <sup>a</sup>		Above base <sup>a</sup>	Below base <sup>a</sup>		Above base <sup>a</sup>	Below base <sup>a</sup>		Above base <sup>a</sup>	Below base <sup>a</sup>
Extracted wood. . . . .	No. 3	Per cent 188.4	No. 5	No. 2	Lbs. per sq. in. 5,770	Per cent 106	No. 1	In.-lbs. per cu. in. 7.97	Per cent 133	No. 7	Per cent 108	In.-lbs. per sq. in. 6.46	
Adjacent control <sup>b</sup> . . . .	3	91.2	5	2	5,420	109	2	5.98	164	6	112	6.70	
Separate control <sup>c</sup> . . . .	3	31.2	5	2	5,890			9.79					

## AIR-DRY WHITE SPRUCE

 $\frac{5}{8}$  x  $\frac{5}{8}$  inch in cross section

Extracted wood. . . . .	3	13.0	103	5	2	9,400	104	3	4	104	9,450	6.16	106	6.85
Adjacent control <sup>b</sup> . . . .	3	13.0				9,070	103	5	2	109	9,900	6.47	112	7.24
Separate control <sup>c</sup> . . . .	3	13.0	106	7	0	9,320						8.16		

<sup>a</sup> The base values are the averages under "Adjacent control."<sup>b</sup> Cut from the outer part of the piece through the center of which water was forced.<sup>c</sup> Cut from the same annual growth rings as the extracted specimens but not adjacent to them.

The method<sup>11</sup> for removing extractives was merely to force cold water through the wood in the direction of the grain, and in detail was as follows:

Green redwood boards  $2\frac{1}{2}$  inches thick, 10 to 12 inches wide, and 6 to 24 inches long were used. A  $\frac{3}{4}$ -inch hole was bored near the middle of the length (fig. 2), into the side grain of the narrow face, and to within 1 inch of the opposite face. A pipe threaded at its end was then screwed in for about 1 inch, after which it was attached to a water main that was under a pressure of about 80 pounds per square inch. Since water moves along the grain much more rapidly than across the grain, the portion of the piece indicated by dotted lines in Figure 2 was the natural course for the water, and hence this portion lost a much larger percentage of extractives than the remainder. Water was allowed to seep through the 6-inch pieces for about 70 days, and the 24-inch pieces for about 120 days. The total amount of water passing through was about the same for the short as for the long pieces, during the test periods, although a slightly greater percentage of extractives was removed from the short ones.

The specimens from the central portion of the board, from which a large part of the extractives had been removed, were compared with specimens taken from near the face of the board. Since these boards were quarter-sawn and the hole was bored into the narrow face, matched specimens came from the same annual rings. Other specimens carefully matched to the treated specimens but not part of the treated piece were also tested as controls. The specimens were from one-half to three-fourths inch square in cross section. Tests of material in both the green and the air-dry condition were made. In series 2 and 3, where both compression-parallel-to-grain and static-bending tests were made, two compression-parallel-to-grain specimens were obtained from the ends of the static-bending specimen after test.

The results of these tests are presented in Table 2.

The amount of extractives remaining in the portion of the redwood pieces leached by water was approximately 5 per cent<sup>12</sup> and the remainder of the board had about 10 per cent. Control pieces carefully matched to the treated piece had an extractive content of approximately 12 per cent, indicating that the outer portion of the treated board lost some extractives while the interior portion lost over half. The strength ratios given in Table 2 are based on the strength of the outer portion of the treated board, and since this material had also lost some extractives, the indicated changes in strength caused by the removal of extractives from the inner portion of the board are probably somewhat less than those that actually occurred.

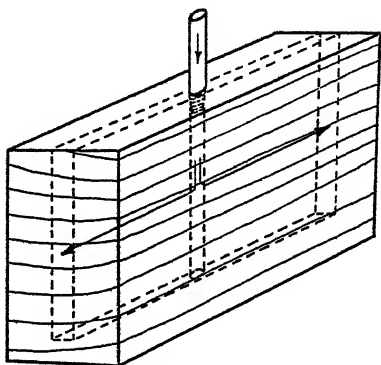


FIGURE 2.—The course of the main flow of water in test material

<sup>11</sup> This method of forcing water through a board was conceived by H. D. Tiemann of the Forest Product Laboratory.

<sup>12</sup> The percentage of extractives is based on the weight of the wood, oven-dry for all specimens.

## RESULTS OF SERIES 1 AND 2 TESTS

When the redwood specimens were tested in a green condition the loss in extractives caused a loss in maximum crushing strength approximately twice that which would normally accompany a like reduction in the amount of wood substance. The loss in modulus of rupture was about the value that would accompany a like loss in the amount of wood substance, while the work to maximum load was decreased even less than would be expected from an equal loss in weight of wood substance. These findings, which in effect are that removal of extractives affected the strength properties inversely from the infiltration of extractives, substantiate the theory that the difference in strength between heartwood and sapwood of redwood is primarily due to extractives.

With air-dry redwood both the maximum crushing strength and the modulus of rupture showed less percentage reduction from the removal of extractives than in green material. Work to maximum load showed a slight increase because of the removal of extractives. These results are in agreement with the tests of air-dry sapwood and heartwood.

The series 1 specimens had a higher proportion of extractives removed than the series 2. Comparing these two series, it may be seen that the maximum crushing strength was lowest for the pieces showing the greatest reduction in weight, indicating that the more of the extractives removed the greater the effect upon the strength.

## RESULTS OF SERIES 3 TESTS

The leaching treatment of white spruce apparently did not decrease the weight of the wood. In fact, the outer portion of the treated blocks was lower in specific gravity than the central portion, through which most of the water undoubtedly flowed. The maximum-crushing-strength and modulus-of-rupture values for both green and air-dry material from the central portion of the treated block were also higher than those for the outer portion, and compared favorably with the carefully matched control specimens that were not part of the treated block. In work to maximum load the central portion of green material was considerably higher than the outer portion, while in the air-dry wood the reverse was true. The tests on white spruce indicate little, if any, effect on the weight and the strength of this wood resulting from the process used for removing extractives.

## EFFECT OF INCREASING THE CONCENTRATION OF EXTRACTIVES THROUGH DRYING

If the removal of extractives affects the strength of redwood, then an increase in concentration should also affect it. Sherrard<sup>13</sup> has shown that when redwood is dried there is a transfer of extractives from the center outward that results in a higher concentration near the surface. Tests were made to compare the effect of this increase in concentration on the strength. Specimens from the outermost part of the block were compared with specimens from the same block and the same annual rings but slightly nearer the center of the piece. The high extractive content decreases very rapidly from the outer

<sup>13</sup> Unpublished data.

surface inward; the first quarter inch from the surface averaged over 12 per cent extractives, while the second quarter inch averaged only 5 per cent. Therefore, to get a large difference in extractives in closely matched pieces small specimens were necessary, and pieces one-fourth by one-fourth inch in cross section were used. Compression-parallel-to-grain and static-bending tests were made, the results of which are shown in Table 3. Specimens from two trees were tested and similar results were obtained for both sets of specimens.

The difference in specific gravity of the first quarter inch of material from the surface compared with the second quarter inch was approximately 6 per cent; this difference was undoubtedly due to the transfer of extractives during drying. In compression-parallel-to-grain tests the outer layer was about 7 per cent higher in strength than the inner one and the modulus of rupture was about 4 per cent higher, while work to maximum load showed little change. Although the differences are not large, the fact that there is a change and that individual specimens were quite consistent in showing a change is further evidence that extractives do affect the strength properties.

#### EFFECT OF ADDING EXTRACTIVES

Another method used for determining the effect of extractives on the strength was to soak small, green, sapwood specimens of redwood in a solution of redwood extractives. The solution, which was expressed from green redwood by the cooperating company<sup>14</sup> that supplied it, had an extractive concentration of 3.83 per cent. Only short specimens 2 inches in length were treated, since the greatest increase in extractive content through soaking naturally occurs at the ends. These short pieces permitted only compression-parallel-to-grain tests.

In addition to the sapwood pieces soaked in a redwood solution, pieces carefully matched for control were soaked in water under conditions otherwise identical, and were then compared with matched untreated controls. The results of the tests are shown in Table 4.

Soaking sapwood specimens of redwood in water had little effect on the specific gravity of the oven-dry wood or on the maximum crushing strength. When soaked in redwood extractives, the specific gravity oven-dry was increased about 10 per cent. The maximum crushing strength was slightly increased, but the change was small in comparison with the change in weight caused by the addition of extractives. These comparisons held for material tested immediately after soaking as well as for that subsequently air-dried.

The relatively small increase in crushing strength as compared with the increase in weight caused by the infiltration of extractives is probably due to the high concentration of extractives near the ends of the specimens and the lower concentration near the center, where practically all of the failures occurred. Although no marked change in strength was shown as a consequence of soaking the specimens in extractives, the fact that some increase resulted and that this increase was fairly consistent is of interest as further evidence that extractives do affect the strength.

<sup>14</sup> The redwood for this investigation was supplied by the California Redwood Association and the solution of extractives by the Little River Redwood Co. of Crannell, Calif.

TABLE 3.—The strength values of the outer portion of virgin redwood timber, the extractive content of which has been increased through kiln drying, compared with those of the interior

## OUTER PART OF KILN-DRIED PIECE

Tree No.	Kind of specimen <sup>a</sup>	Tests	Cold-water extractives	Compression parallel to grain										Static bending																	
				Specific gravity					Maximum crushing strength					Specific gravity					Modulus of rupture					Work on maximum load							
				Moisture content					Adjusted to 10 per cent moisture content					Adjusted to 10 per cent moisture content					Adjusted to 10 per cent moisture content					As tested							
				Value	Ratio of <i>k</i> to <i>m</i>	<i>k</i> speci- mens— Below <i>m</i>	<i>k</i> speci- mens— Above <i>m</i>	Per cent	Lbs. per sq. in.	Value	Ratio of <i>k</i> to <i>m</i>	<i>k</i> speci- mens— Below <i>m</i>	<i>k</i> speci- mens— Above <i>m</i>	Per cent	Lbs. per sq. in.	Value	Ratio of <i>k</i> to <i>m</i>	<i>k</i> speci- mens— Below <i>m</i>	<i>k</i> speci- mens— Above <i>m</i>	Per cent	Lbs. per sq. in.	Value	Ratio of <i>k</i> to <i>m</i>	<i>k</i> speci- mens— Below <i>m</i>	<i>k</i> speci- mens— Above <i>m</i>	Per cent	Lbs. per sq. in.	In.-lbs. per cu. in.	Ratio of <i>k</i> to <i>m</i>		
																														Per cent	No.
66	<i>k</i>	8	13.67	9.5	0.437	6,470	9,360	7	1	10.6	7	3	7.6	0.446	8	0	11,800	12,000	6	2	10.00	8	2	12.88	10	5	5	5	102	10	7
	<i>k</i>	10	10.93	7.2	0.454	7,600	7,000	7	1	7.6	7	1	7.6	.481	7	3	13,140	12,450	6	2	12.88	8	2	12.88	14	4	4	4	11.44	10	7
Total		18	12.30	8.3	.446	7,040	6,680	14	4	9.1	14	4	9.1	464	107	15	3	12,470	12,230	14	4	11.44	14	4	11.44	104	4	4	102	10	7
Average			12.30	8.3	.446	7,040	6,680	107	4	9.1	107	4	9.1	464	107	3	3	12,470	12,230	104	4	11.44	104	4	11.44	104	4	4	102	10	7

## INNER PART OF KILN-DRIED PIECE

6.	<i>m</i>	8	6.83	10.7	0.395		5,700	5,820				11.7	0.395				11,100	11,550					9.89	
	<i>m</i>	10	5.02	7.8	.468		7,170	6,720				8.1	.475				12,450	11,900					12.68	
Total	Average	18	5.92	9.2	.426		6,440	6,270				9.9	.435				11,780	11,730					11.28	

<sup>a</sup> Specimens from the surface of kiln-dried timber, which are high in extractives, are designated k. Specimens adjacent to the outside pieces, which are considerably lower in extractives, are designated m.

TABLE 4.—The maximum crushing strength of virgin redwood sapwood, impregnated with redwood extractives, compared with that of unimpregnated wood

## GREEN WOOD

Condition of specimen	Matching <sup>a</sup> of specimens	Tests	Moisture content	Specific gravity			Maximum crushing strength				
				Value	Ratio of treated to control specimen	Impregnated specimens—		Value	Ratio of treated to control specimen	Impregnated specimens—	
						Above controls	Below controls			Above controls	Below controls
		Number	Per cent	Per cent	Number	Number	Per cent	Number	Number		
Soaked in water	A.....	6	38.6	0.322	101	3	2,700	102	3		
Green.....	do.....	6	35.8	.320	111	0	2,650	b 102	2		
Soaked in extractives	B.....	6	61.0	.356	111	6	2,770	b 102	4		
Green.....	do.....	6	35.7	.322	111	4	2,720	b 102	2		

## AIR-DRY WOOD

Condition of specimen	C.....	6	9.5	0.342	101	2	4	4,790	2						
										6	9.6	.342	101	4	4,790
D.....	6	9.2	.370	109	6	0	5,270	3							
Soaked in water and air dried.....	do.....	6 <td>9.5<td>.340<td>109<td>6<td>0<td>5,100<td>3</td></td></td></td></td></td></td>	9.5 <td>.340<td>109<td>6<td>0<td>5,100<td>3</td></td></td></td></td></td>	.340 <td>109<td>6<td>0<td>5,100<td>3</td></td></td></td></td>	109 <td>6<td>0<td>5,100<td>3</td></td></td></td>	6 <td>0<td>5,100<td>3</td></td></td>	0 <td>5,100<td>3</td></td>	5,100 <td>3</td>	3						
Adjusted for moisture.....	do.....	6 <td>9.8<td>.340<td>109<td>6<td>0<td>5,080<td>3</td></td></td></td></td></td></td>	9.8 <td>.340<td>109<td>6<td>0<td>5,080<td>3</td></td></td></td></td></td>	.340 <td>109<td>6<td>0<td>5,080<td>3</td></td></td></td></td>	109 <td>6<td>0<td>5,080<td>3</td></td></td></td>	6 <td>0<td>5,080<td>3</td></td></td>	0 <td>5,080<td>3</td></td>	5,080 <td>3</td>	3						
Air dried.....	do.....	6 <td>9.8<td>.340<td>109<td>6<td>0<td>5,080<td>3</td></td></td></td></td></td></td>	9.8 <td>.340<td>109<td>6<td>0<td>5,080<td>3</td></td></td></td></td></td>	.340 <td>109<td>6<td>0<td>5,080<td>3</td></td></td></td></td>	109 <td>6<td>0<td>5,080<td>3</td></td></td></td>	6 <td>0<td>5,080<td>3</td></td></td>	0 <td>5,080<td>3</td></td>	5,080 <td>3</td>	3						

<sup>a</sup> All specimens were intermatched, but those carrying the same letter were matched somewhat more closely than the others.<sup>b</sup> Both the relatively high increase in weight and the small increase in strength resulting from the injection of extractives may be caused by the concentration of the extractives near the ends of the pieces. The failure of extractive-impregnated specimens occurred consistently near the middle of the length.



## CONCLUSIONS

The several methods of investigation employed show that extractives affect the strength properties of the species of wood examined. The extent of this effect depends upon the amount of extractives, the species of wood, the moisture condition of the piece, and the mechanical property under consideration. Of the properties considered, the compressive strength parallel to the grain appears to show the greatest increase because of the normal infiltration of extractives in the change of sapwood into heartwood, the modulus of rupture is next, and shock resistance is least. In fact, under some conditions the shock resistance appears to be actually lowered by extractives. That extractives may affect different species differently is indicated by the fact that they change the strength of western red cedar less than the strength of black locust, although black locust has a smaller percentage of extractives. When the mechanical properties of wood do not vary regularly with the specific gravity, the presence of extractives or irregularity in their content may be at least a contributing factor.

## SUMMARY

Extractives, which occur in the wood of many species, are especially abundant in redwood, western red cedar, black locust, and a few others. The three species mentioned are also relatively high in certain strength properties, for the amount of wood substance they contain, and no study of this peculiarity has heretofore been made at the Forest Products Laboratory. The tests reported here were made for the purpose of determining whether a relation exists between the extractive content of wood and its strength.

Four distinctly different test methods were used, and all of them indicated strongly that extractives do affect the strength of wood. Of the properties studied, compressive strength parallel to the grain appears to be increased most by the presence of extractives, bending strength as measured by modulus of rupture next, and shock resistance least.

# STUDIES ON SCLEROTIA AND MYCELIAL STRANDS OF THE COTTON ROOT-ROT FUNGUS<sup>1</sup>

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## INTRODUCTION

The discovery of a true sclerotial stage in the life history of the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, was reported by King and Loomis<sup>2</sup> from laboratory observations made at Sacaton, Ariz., in September, 1928. The relationship of these hold-over bodies to the disease was further confirmed by Neal,<sup>3</sup> who first reported the discovery of sclerotia under natural conditions in the soil, at Greenville and San Antonio, Tex., in 1929; and by Taubenhaus and Ezekiel,<sup>4</sup> who have also reported finding sclerotia under field conditions in Texas. During the season of 1930 the writers found them in great numbers in several areas in Arizona, but they occurred only in limited parts of the infected spots.

In order to determine the relationship of these structures to the dissemination of the disease and to its maintenance for long periods in the soil, an investigation of their properties was undertaken at the United States field station at Sacaton. Inoculation experiments were conducted and various tests were made to ascertain the length of time the sclerotia remained viable under different conditions and the strength of disinfecting solutions required to kill them.

To permit of uniform comparison in all the tests of sclerotia, only well-developed simple individuals were used. On account of the extreme variability in size and form of the compound sclerotia and the short life and lack of vigor of the smallest simple individuals, these types were not selected.

## INOCULATION STUDIES

Since it was found that the sclerotia germinated readily, producing the characteristic root-rot mycelium, it seemed reasonable to suppose that they might be able to infect healthy plants when brought in contact with their roots. Inoculation experiments showed that this was a correct assumption. Clusters of sclerotia from pure cultures were placed in contact with the taproots of 17 cotton plants grown in disease-free soil in iron drums, and 2 of these plants were killed. The same treatment on 55 cotton plants grown under field conditions, where no root rot had ever been in evidence, resulted in the infection of 9 plants; but only one of these died before the end of the season. Successful inoculations with sclerotia have been reported also by D. C. Neal from studies carried on at the United States San Antonio Field Station, San Antonio, Tex.

<sup>1</sup> Received for publication Dec. 11, 1930; issued June, 1931.

<sup>2</sup> KING, C. J., and LOOMIS, H. F. FURTHER STUDIES ON COTTON ROOT ROT IN ARIZONA, WITH A DESCRIPTION OF A SCLEROTIUM STAGE OF THE FUNGUS. *Jour. Agr. Research* 39: 641-676, illus. 1929.

<sup>3</sup> NEAL, D. C. THE OCCURRENCE OF VIABLE COTTON ROOT-ROT SCLEROTIA IN NATURE. *Science (mis.)* 70: 400-410. 1929.

<sup>4</sup> TAUBENHAUS, J. J., and EZEKIEL, W. N. OVERWINTERING AND SPREAD OF PHYMATOTRICHUM ROOT ROT. (Abstract) *Phytopathology* 20: 117. 1930.

On the other hand, 13 of 62 field-grown cotton plants were killed when inoculated with pure cultures of mycelia grown on dead root tissues, and 16 of 33 plants were killed when the inoculum consisted of sections of cotton roots from plants that had recently wilted from effects of the disease in the field. It was found in laboratory experiments that the mycelium from a germinating sclerotium was capable of extending a distance of 14 cm. through an unsterilized soil that was free from roots. These bodies, therefore, doubtless are capable of infecting plants without being in direct contact with their roots.

A further development from the inoculation studies conducted at Sacaton in 1929 was the success obtained in killing healthy plants with a pure culture of mycelia on dead cotton roots taken from a jar that had remained undisturbed in the laboratory for 11 months and in which no sclerotia were observed.

#### EFFECTS OF AIR DRYING, IMMERSION IN WATER, AND HEAT ON GERMINATION OF SCLEROTIA

In a previous paper <sup>5</sup> King and Loomis have reported that when relatively fresh sclerotia are removed from cultures, surface sterilized from 30 to 45 seconds in a 1:1,000 mercuric chloride solution, and incubated at about 25° C. on a suitable medium, 95 to 100 per cent of them may be expected to germinate.

Sclerotia that formed October 26, 1928, in a jar culture, were left undisturbed until November 7, 1929. On the latter date 46 of them were removed, placed on steamed rice in Petri dishes, and incubated at 25° C. After about 48 hours it was found that 42 of the 46 had germinated. To test the effects of drying, numbers of fresh sclerotia were removed from pure cultures and exposed to drying in the air for varying lengths of time. After drying they were placed on a culture medium and incubated. The germination results are shown in Table 1.

TABLE 1.—Germination of sclerotia of *Phymatotrichum omnivorum* after drying in air for various periods

Period of drying (minutes)	Sclerotia dried in air		Period of drying (minutes)	Sclerotia dried in air	
	Total	Germinated		Total	Germinated
	Number	Number		Number	Number
6.....	10	10	30.....	20	16
15.....	20	20	45.....	20	14
18.....	10	10	60.....	10	3
21.....	11	11	75.....	10	0
24.....	10	9	90.....	10	0

It will be noted that exposure to the air at room temperature for about 75 minutes was sufficient to kill these simple sclerotia. A longer period of drying would no doubt be required to destroy the large, compound masses of sclerotia that sometimes occur in cultures and in nature.

The results obtained by drying simple sclerotia in a desiccator containing sulphuric acid were similar to those shown above. All the sclerotia exposed to such drying for 60 minutes failed to germinate.

It has been observed in repeated tests that sclerotia remain viable for long periods of time when immersed in distilled water. In one test, a quart-jar culture of sand and dead cotton roots, inoculated July 9, 1929, and containing a large number of sclerotia that had developed between July 18 and 24, was filled with distilled water to a point above the sand and roots and stored in the laboratory. Small numbers of sclerotia were removed from time to time thereafter for germination tests. In all the tests made during the first two or three months nearly all the sclerotia germinated. One test, made October 24, 92 days after the immersion treatment, resulted in the germination of 13 of the 16 sclerotia removed, or 81 per cent. However, a later test, made 121 days after the culture was submerged, resulted in the germination of only about 20 per cent of the sclerotia, and many showed signs of disintegration.

An indication of the sensitiveness of the sclerotia to heat was obtained by placing fresh sclerotia in test tubes of distilled water, which were then immersed in water baths with controlled temperatures. The baths in which the sclerotia were immersed for 15 minutes were maintained at temperatures ranging by 1-degree stages from 40° to 47° C. No definite loss of vitality was shown by immersion in the 40°, 41°, 42°, and 43° baths; but a distinct decrease in viability was evident after exposure to the next two higher temperatures (Table 2), and no sclerotia remained alive after immersion for 15 minutes in the 46° or 47° baths.

TABLE 2.—*Viability of sclerotia immersed for 15 minutes in uniform-temperature baths of 40° to 47° C.*

Temperature maintained (°C.)	Sclerotia tested	Sclerotia viable	Temperature maintained (°C.)	Sclerotia tested	Sclerotia viable
	<i>Number</i>	<i>Number</i>		<i>Number</i>	<i>Number</i>
40.....	15	14	44.....	40	24
41.....	15	13	45.....	40	14
42.....	15	14	46.....	25	0
43.....	30	27	47.....	10	0

In later experiments quart fruit jars were partly filled with moist sand, and a few sections of infected cotton roots about one-half inch in diameter and some simple sclerotia were placed in the center of the mass of sand. A thermometer was then inserted with the bulb in contact with the fungus material. The jars were placed in the water baths, where they were slowly heated until the temperature of the center of each jar reached the desired point, as indicated by the thermometer. The jar was then immediately removed and allowed to cool. The maximum temperature at the center of the jars ranged from 38° to 52.5° C. In most cases the maximum temperature was held from two to four minutes before the center of the jar began to cool. When the root sections and sclerotia were tested, it was found that a maximum temperature of about 43° was sufficient to kill the sclerotia, while a maximum temperature of about 51° was required to kill the mycelium on and in the root tissues.

## EFFECT OF DISINFECTANTS ON GERMINATION OF SCLEROTIA

As solutions of formaldehyde have been used with a fair degree of success for several years in experimental control or eradication of root-rot infection in field crops, it seemed advisable to ascertain the susceptibility of the sclerotia to these solutions. A preliminary test was made under laboratory conditions to determine the strength of solution that would likely prove best suited for application in the field. Individual sclerotia of average size in lots of 10 were immersed for 30 minutes in solutions ranging from 1 per cent commercial formalin to as low as one-sixteenth of 1 per cent. In two tests, all sclerotia exposed in solutions of one-half of 1 per cent or less germinated, while of those tested in the 1 per cent solution, 2 of a total of 10 grew in the first trial and 3 of a total of 10 in the second. As might be expected, later tests indicated that when the period of immersion was lengthened the viability of the sclerotia was reduced.

To determine the effects of formaldehyde on sclerotia under conditions similar to those in the field, cylindrical cores of soil 1 foot in diameter and 1-foot deep were removed intact from the field in iron tubes. Commercial formalin solutions of 1 per cent and 1½ per cent were added to these cylinders of soil until they were saturated, and the excess solution was allowed to drain off. At intervals of 24 hours during the first four days and on the sixth day after disinfection, 10 fresh sclerotia, inclosed in slender cylinders of filter paper containing fine, dry sand, were introduced into each core of soil, and the treated soil was packed around them. The sclerotia were allowed to remain in the soil for three hours and were then removed, washed in distilled water, and tested for germination. All of the sclerotia subjected to this treatment failed to germinate.

Sections of infected cotton roots, about one-half inch in diameter, wrapped in filter paper, were inserted in the soil in the same manner and at the same time as the cylinders containing sclerotia. These were allowed to remain in the treated soil for 12 hours and were then removed and placed in moist chambers. Those placed in the soil during the first four days after treatment showed no fungus growth, but a growth of mycelium developed on those inserted on the sixth day, indicating that the strength of the formalin was not being maintained.

In order to study the lethal effects of formaldehyde gas on the fungus under various forms and conditions, an experiment was conducted as follows: Glass tubes 3 feet long and 1 inch in diameter were filled with moist sterile sand and inoculated at one end with a small section of infected root tissue. When the growing strands had advanced several inches through the sand, additional sections of infected root tissue and a number of sclerotial clusters of various sizes were inserted in the sand at various intervals in advance of the growing strands. A flask containing a solution of 1½ per cent formalin was then attached to one end of each tube and an aspirator was connected to the other end. The solution of formalin was heated slightly and the vapor was drawn through the tubes in which the fungus and sclerotia were exposed. Another set of tubes was set up in like manner, but water instead of the formalin solution was used as the source of vapor.

It was noted that the elongation of the strands in the tubes ceased soon after they were exposed to the formaldehyde gas, but was not

interrupted in the control tubes. At the end of 21 hours the sclerotia and root tissues were removed from both sets of tubes and placed in moist chambers and incubated. A growth of mycelium resulted from all the clusters of sclerotia and from all the root tissues removed from the control tubes, but no growth came from the sclerotia and tissues exposed to the formaldehyde gas. (Fig. 1.)

In another test, sclerotia in groups of 10 individuals were immersed for 30 minutes in solutions containing 1 part mercuric chloride to 1,000, 2,000, 3,000, 4,000, and 5,000 parts of water, and were tested for viability on steamed rice. None of the 10 sclerotia that had been placed in each of the two highest concentrations germinated, but of those subjected to the three lower concentrations, 3 or 4 in each culture of 10 developed growth.

As a further test other groups, also of 10 sclerotia, were immersed in a 1:1,000 mercuric chloride solution for periods of one-half, 1, 2, 3, 4, and 5 minutes. They were then removed, rinsed with sterile water, and tested for germination. In the four shortest periods of immersion the number germinating was 5, 2, 4, and 1, respectively, while no sclerotia germinated after being submerged for 4 or 5 minutes.

#### RELATION OF PERIODIC DEVELOPMENT OF SCLEROTIA TO LONGEVITY OF THE FUNGUS

In several cultures it has been observed that old clusters of sclerotia are sometimes regenerated by a budding process in which new sclerotia are formed. (Fig. 2.) Occasionally old strands may develop swellings and produce a few sclerotia in the same direct manner as new and actively growing strands, but more often the late-developed sclerotia appear to be an excrescent growth from old sclerotial structures. The growth of the nodular masses is sometimes more or less continuous for a long time, but in other cases there appears to be a period of quiescence in the entire culture which may last for several weeks or months. The renewed growth on the strands or sclerotia clusters is made evident by its whitish or pearly appearance in contrast to the dark or reddish-brown color of the old growth. The new portions when detached and tested show a much higher proportion of germination than the older portions.

TABLE 3.—Number of days between date of inoculation and dates on which earliest and latest additions of growth were observed in the formation of sclerotial accretions in cultures of *Phymatotrichum omnivorum*

Culture No.	Culture inoculated	Period after inoculation		Culture No.	Culture inoculated	Period after inoculation	
		First sclerotia observed	Last addition to old sclerotial clusters			First sclerotia observed	Last addition to old sclerotial clusters
		Days	Days			Days	Days
A-1.....	Sept. 20, 1928	37	240	145-1.....	Aug. 29, 1929	15	102
B-1.....	.....do.....	41	132	170-2.....	Sept. 11, 1929	12	242
9-2.....	Jan. 22, 1929	21	117	232-1.....	Nov. 6, 1929	19	185
16-1.....	Jan. 25, 1929	18	114	243-1.....	Nov. 18, 1929	11	99
17-1.....	.....do.....	18	114				



FIGURE 1.—Effect of formaldehyde gas on sections of cotton roots infected with *Phymatotrichum omnivorum*. Natural size. A, Root sections 7 days after removal from tubes of sand where they were exposed for 21 hours to a current of vapor from a 1½ per cent formalin solution. No root-rot strands have developed from the root sections. B, Root sections 7 days after removal from control tubes of sand where they were exposed for 21 hours to the vapor from water. Root-rot strands have originated from each root section



FIGURE 2.—New growth of root-rot sclerotia developed by a process of budding on old, compound sclerotial structures 230 days after the first portions of the mass were observed. The dotted line defines the limits of the old masses of sclerotia, and the arrow designates the new formations of growth.  $\times 5$



It has not been determined how long this periodic production of new sclerotial material may continue, but it is not unreasonable to suppose that the process might be carried to the stage of a third crop, or even beyond. Under field conditions such a process would allow viable sclerotia to be present in the soil for long periods in the absence of the active hyphal forms of the fungus, so that practices of clean cultivation or the planting of nonsusceptible crops could not be relied upon to eradicate or control the disease.

Data on a number of cultures in which old sclerotia developed new portions are shown in Table 3.

The culture numbered 9-2 in Table 3 was prepared January 22, 1929, in a quart fruit jar containing moist sand and dead cotton roots, and cultures 16-1 and 17-1 were prepared in like manner on January 25. On February 12 these three cultures on which development of sclerotia had begun, three other fresh cultures which contained no sclerotia, and an old culture containing a single sclerotium were placed in a wooden box and buried in the field, so that the tops of the cultures were about 1 foot beneath the surface of the soil. On May 19 the cultures were removed, and the three named above, which contained new sclerotia when buried, showed recent development of sclerotia. The four other cultures showed no indications of recent growth. The lowest air temperature recorded near by during the period of exposure in the soil was 26° F. A germination test was made to determine the viability of the old dark-colored sclerotia, the reddish-brown type of intermediate age, and those newly developed, which were white or grayish in color, in the three cultures that contained the new sclerotia. None of the dark-colored individuals germinated, but a large proportion of the reddish-brown group and all the recently developed sclerotia were found to be viable.

#### GROWTH RATE OF STRANDS IN GLASS TUBES

Further studies were made on the growth of mycelial strands through long glass tubes, 1 inch in diameter, containing sand and soil. The daily progress through the tubes of sand was similar to that previously reported,<sup>6</sup> but longer tubes were used. Disease-free moist soil, removed from field plots adjacent to root-rot spots, was placed in some of the tubes without sterilization and a pure culture of the fungus introduced at one end of the tubes on a small piece of dead cotton root. The growth and behavior of the strands which resulted were similar to that noted in previous experiments with sterile soil. The daily measurements of strand growth were based on the elongation of the hyphae that were visible near the surface of the glass tube. A careful study of the behavior of the fungus in such tubes in previous experiments had shown that when moisture conditions were favorable the advancing hyphae made progress among the sand particles at about the same rate as along the glass surface.

In one test a glass tube 15 feet long, filled with moist, sterile sand, was used, and the fungus was introduced at one end on a piece of dead cotton root on June 19. At intervals of approximately 2½ feet throughout its length, spaces about 2 inches long in the tube were filled with pieces of sterilized dead cotton roots. The fungus began to progress through the tube from the inoculum on June 21, and on

<sup>6</sup> KING, C. J., and LOOMIS, H. F. Op. cit. (See footnote 2)

June 26 a crop of sclerotia began to form on mycelial growth that developed between June 22 and 24. The next deposit of sterilized roots was completely enveloped on July 15, and on July 27 a second formation of sclerotia was observed developing on growth that had formed from July 19 to July 25, inclusive. The third deposit of cotton roots was enveloped on August 11, and on August 18 a third formation of sclerotia was noted on that part of the strands which developed between August 12 and 16. The next deposit of roots was passed September 2 without sclerotia being formed, but following the passage of the last deposit, about October 10 or 11, a single

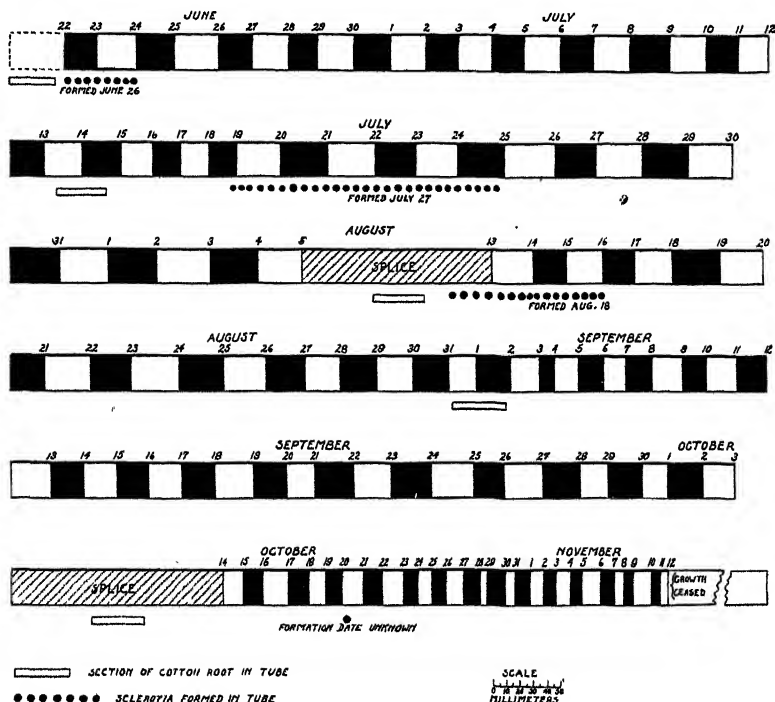


FIGURE 3.—Daily elongation of root-rot strands from June 22 to November 12, 1929, through a 15-foot glass tube, 1 inch in diameter, containing sand in which pieces of cotton roots were placed at intervals of approximately  $2\frac{1}{2}$  feet

sclerotium was formed on a strand developed October 20, although the date of its formation was not noted.

Elongation of the strands in this tube terminated on November 12, the strands having traversed a distance of 3,185 mm. in the 149 days, an average daily growth of a little more than 21 mm. A maximum daily growth of 37 mm. occurred twice during the test. A slight increase in the rate of elongation of the strands was noted several days after their terminal growth had passed the stored root tissues, and the increased rate was maintained even during the period when new sclerotia were being developed. A diagram of the daily elongation of the fungus in this tube, the location of the deposits of food, and the points at which sclerotia were formed is shown in Figure 3.

It appears significant that the strands in this and other tests began the development of sclerotia soon after having made contact with a

fresh food supply. Sclerotia have been observed in tubes of glass beads as far as 25 cm. from the nearest food material; but, as the strands become noticeably smaller on extending much farther than this, it seems probable that a diminution in the development of sclerotia may be expected as the distance is increased.

On the basis of early observations on the behavior of the fungus in alfalfa fields, it was reported that the mycelium showed a tendency to spread centrifugally from initial points of infection and only occasionally made an immediate reinvasion to attack plants sown in the area behind the advancing zone of infestation.<sup>7</sup> An effort was made to determine whether the mycelium, in its movement through the sand or soil, left anything in the nature of a toxic residue that would inhibit a second or third passage of the strands. Two glass tubes 3 feet long, containing sand that was permeated with root-rot hyphae and strands developed in August, 1928, were selected for the test. One of these was reinoculated on August 9 and the other on August 14, 1929, with infected cotton root tissues introduced at the same end of each tube as previously. By September 2 the strands had grown completely through the tube inoculated on August 14, a distance of 594 mm. in 19 days. By September 3 the mycelium had completed its passage through the tube inoculated on August 9, having grown a distance of 640 mm. in 25 days. The average daily growth in these tubes was 31.3 mm. a day. The same tubes were inoculated for the third time, without sterilization, on September 13, after the sand had been shaken to break up and obscure the old mycelia so that the growth of the new strands might be observed and measured more easily. The passage of the strands through the entire length of one tube was completed on October 24, and through the other on October 28. During the period when this third test was being conducted the temperatures were considerably lower than during the previous tests, and this factor, doubtless, accounted for the slower rates of growth, the average of which was 16 mm. and 13.2 mm. per day in the respective tubes.

In the light of the behavior of the fungus in these tubes it would not appear that the root-rot mycelium leaves any substance in the soil that is toxic to new fungus growth.

One interesting result of breaking up the strands by shaking the contents of the tubes before the third inoculation was the development of numerous hyphae from the ends of many of the broken fragments of the old strands. Other broken pieces of coarse strands, removed from sand in old cultures, were transferred to culture tubes containing root tissues, which were promptly enveloped by the resulting new growth. This behavior is in accord with Duggar's<sup>8</sup> belief that the strands are sclerotial in character. There appears, however, to be a great diversity in the size and structure of strands, which may govern their longevity, some forms in culture and in nature remaining viable much longer than others. The larger sclerotia appear to be longer lived than any of the strand forms so far observed.

In connection with the study of the mycelial strands in long glass tubes containing moist sand and soil, tests were made in which 2-mm.

<sup>7</sup> KING, C. J. HABITS OF THE COTTON ROOT-ROT FUNGUS. *Jour. Agr. Research* (1923) 26: 405-418, illus. 1924.

<sup>8</sup> DUGGAR, B. M. THE TEXAS ROOT-ROT FUNGUS AND ITS CONIDIAL STAGE. *Ann. Missouri Bot. Gard.* 3: 11-23, illus. 1916.

glass beads were substituted for the sand and soil so as to insure the complete absence of organic matter other than the inoculum. Sterile water was added until the beads were saturated, and the gravitational water was then drained away, leaving only film moisture, after which the tubes were inoculated with the fungus on sections of cotton roots. The rate of elongation of the strands through the beads was considerably slower than through the sand, but in some cases the growth continued for more than a month, and in one 2-foot tube the mycelium traversed a distance of 511 mm. in 31 days, the most rapid growth in a single day being 23 mm. In one of the six tubes of beads included in the test, sclerotia developed in great numbers on the strands throughout a distance of 250 mm. from the inoculum, and in one place their formation was almost continuous for a distance of 80 mm. A part of this tube containing a chainlike formation of sclerotia is shown in Figure 4, A, while a magnified view of a part of this formation is shown in Figure 4, C. A similar view of a section of another continuous formation in the same tube is shown in Figure 4, B.

The inoculum was removed from one tube of glass beads after the strands had grown a distance of 196 mm., and on the second day following this removal the growth rate slackened considerably; but the retarded growth was maintained for 13 days, during which time the strands extended a distance of 84 mm.

#### VARIATIONS IN VIGOR OF DIFFERENT ISOLATIONS OF THE ROOT ROT FUNGUS

Observations made on a group of 50 cultures, 10 of which were derived from a Texas type culture, 19 from an Arizona type isolated in 1929, and 21 from an Arizona isolation made in 1928, showed that the average number of days required for the first sclerotia to appear after inoculation was 8.8, 11.3, and 13.1 days, respectively.

Nearly all the sclerotia that developed in cultures containing cotton root tissues during September and October, 1928, were derived from a culture of the fungus that had been isolated the previous July. Early in 1929 cultures were obtained from two sources in Texas. One of these produced a vigorous growth of mycelium in all transfers, and on cotton root tissues almost invariably began to develop sclerotia within a few days. Transfers from the other culture developed very slowly, and no sclerotia were produced, although the fungus was kept alive for several months. It was reported that this culture had been maintained on artificial culture media for a long period.

Several new isolations from different areas of infection at Sacaton were made during the summer of 1929, and some of these showed even greater vigor and developed sclerotia more rapidly and abundantly than the isolation made the previous year. Some of the most rapidly growing cultures developed sclerotia within six days after the inoculum material was introduced into the containers, while in other cultures 16 days sometimes elapsed before they began to form. In some cultures, not included in the comparative-vigor tests, periods of 40 to 80 days have been known to elapse between inoculations on dead root tissue and the formation of sclerotia. In one culture inoculated with a sclerotium, 84 days elapsed before new sclerotia were produced.

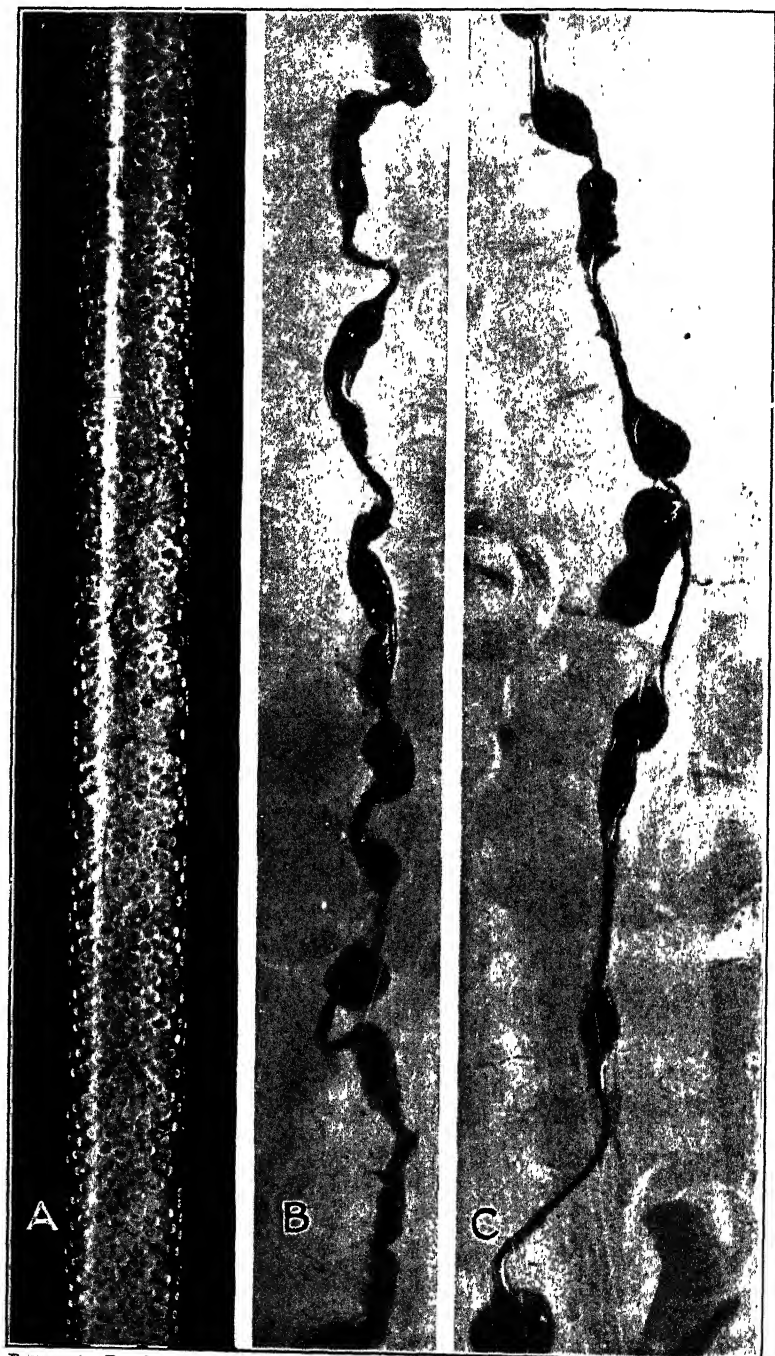


FIGURE 4.—Development of sclerotia on strands of *Phymatotrichum omnivorum* grown in tubes of moistened glass beads. A, Chainlike formation of sclerotia on strands in glass beads over 15 cm. from the root-section inoculum, which was the only food material in the tube (natural size); B, section of one of the strands from this tube, showing almost continuous formation of sclerotia ( $\times 10$ ); C, section of a portion of the strand visible in A, showing an almost continuous series of sclerotia ( $\times 10$ ).

In 1929, isolations were made from several areas in cotton fields where the disease was becoming increasingly destructive, and from other areas where the extent of damage had been decreasing for several years. The mycelium in cultures derived from the areas where the disease was most active appeared to be more vigorous than that from areas where the fungus was less destructive, but no definite conclusions could be drawn on account of the small number of cultures under observation.

The difference in behavior of cultures prepared from recent and from old isolations suggests that the fungus may decline in vigor after being maintained in the laboratory for many months.

#### SUMMARY

Inoculation experiments with sclerotia of the cotton root-rot fungus, *Phymatotrichum omnivorum*, showed that the disease could be communicated to healthy plants by these structures when stimulated by proper conditions.

Air drying for  $1\frac{1}{4}$  hours in an open room or in a desiccator or immersion for 15 minutes in hot water at  $46^{\circ}$  C. was found sufficient to kill individual sclerotia.

Sclerotia immersed in distilled water were 81 per cent viable after 92 days, but after 121 days in water only about 20 per cent germinated.

When simple sclerotia and sections of infected cotton roots one-half inch in diameter were placed in the center of quart jars filled with sand and the jars immersed in water, a maximum temperature of about  $43^{\circ}$  C. maintained for two to four minutes was required to kill the sclerotia, while a temperature of about  $51^{\circ}$  was required to kill the mycelium on and in the root tissues.

Individual sclerotia were killed by a 1 per cent formalin solution and by a 1:2,000 mercuric chloride solution in about 30 minutes and by a 1:1,000 mercuric chloride solution in 4 to 5 minutes.

Sclerotia that were buried for 3 hours in large cores of undisturbed soil from 1 to 6 days after treatment of the soil with 1 per cent and  $1\frac{1}{2}$  per cent formalin solution were killed, and the mycelium on sections of infected cotton roots one-half inch in diameter, buried in like manner for 12 hours in the treated soil, was killed during the first 4 days after the formalin treatment, but that on the roots inserted the sixth day remained alive.

Root-rot sclerotia, exposed strands, and active mycelium on cotton root tissues, placed in glass tubes of sand, were killed when exposed for 21 hours to the gas liberated from a  $1\frac{1}{2}$  per cent formalin solution.

New sclerotia may be developed periodically in old cultures by a budding process on the surfaces of old sclerotial clusters, or directly from old strands, five or six months after the first sclerotia appear. It is indicated that the life of the fungus may be prolonged by this process.

In one experiment, root-rot strands during 149 days grew a distance of 3.2 meters (10.5 feet) through a long glass tube which contained moist sand with small deposits of dead root tissues placed at intervals of  $2\frac{1}{2}$  feet.

It was observed in this and other tests that there was a tendency for sclerotia to develop on the strands soon after they had advanced beyond a fresh food supply. Their development was not immedi-

ate on the new filaments, but usually they formed continuously for several days on the increments of strand growth several days old, between the food supply and the advance hyphae.

The ability of the strands to grow through long tubes of sand three times in succession without resterilization of the sand indicated that no toxic substances were left from the mycelium in the soil to interfere with subsequent growth when reinfected.

It was apparent that some types of root-rot strands are sclerotial in character and under favorable conditions remain viable for long periods.

In a 2-foot glass tube containing only small glass beads and distilled water, root-rot strands advanced a distance of 511 mm. in 31 days from the inoculum placed at one end and developed sclerotia in great numbers over a distance of 250 mm.

Some isolations of the fungus differed from others in vigor of growth and in the ability to develop sclerotia in great numbers. It is possible that differences in the behavior of spots of infection in the field may be explained by such variations.

There are some indications of staling in old root-rot cultures that have been maintained for long periods on artificial media.

# DISPERSION OF SOILS BY A SUPERSONIC METHOD<sup>1</sup>

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## INTRODUCTION

In attempts to remove the colloid from a soil quantitatively there are always evidences that the soil residue still contains some colloidal material. This fact is indicated by determinations of the heat of wetting, the absorption of water vapor, dye, or ammonia, or by microscopic examination of the noncolloidal fraction.<sup>3,4</sup> The colloid can be removed most readily, and probably most completely, by subjecting the soil sample to chemical treatments. In the mechanical analysis of soils by the international method, which is designed to give complete dispersion of the colloidal material, the sample is treated successively with hydrogen peroxide and hydrochloric acid, then washed with distilled water, and finally shaken with an alkaline deflocculent. It seems likely that the colloid extracted from a soil sample so treated would differ in physical and chemical properties from the colloid obtained from soil material dispersed by purely mechanical methods. It is evidently highly desirable to find some method of extracting colloids from soils as completely as possible without resort to chemical treatment, even though the extracted colloid may show alteration in properties due to the mechanical methods of dispersion.

When the remarkable dispersive effects produced by supersonic waves were first announced by Wood and Loomis<sup>5</sup> it was thought that these waves might be used to produce a greater dispersion of soil material than had hitherto been obtained by either physical or chemical means. Accordingly soil samples were submitted to the Loomis laboratory for test, and later, through the courtesy of Mr. Loomis, the supersonic equipment installed in his laboratory was placed at the disposal of the writer for further tests of the dispersive effects of supersonic waves on soil material. As a consequence of these favorable preliminary trials somewhat similar apparatus was installed in this bureau for use in making a comparison of the amount of colloid extractable by supersonic dispersion with that extracted by the hitherto most successful method of mechanical dispersion.

## APPARATUS

The high-frequency, high-intensity waves of the longitudinal sound-wave type used in this investigation, which are called supersonic waves, were produced by a piezoelectric quartz crystal. Such a

<sup>1</sup> Received for publication Feb. 2, 1931; issued June, 1931.

<sup>2</sup> Acknowledgment is gratefully made of the assistance of Capt. E. G. Oberlin and members of the staff of the Naval Research Laboratory in designing and constructing the supersonic driver and in furnishing the piezoelectric quartz crystals; and of the assistance of Dr. C. H. Kunsman, of Fertilizer and Fixed Nitrogen Investigations, Bureau of Chemistry and Soils, in permitting the use of a 1,500-watt radio transmitter and other equipment, as well as in supplying laboratory space for conducting this investigation.

<sup>3</sup> GILE, P. L., MIDDLETON, H. E., ROBINSON, W. O., FRY, W. H., and ANDERSON, M. S. ESTIMATION OF COLLOIDAL MATERIAL IN SOILS BY ADSORPTION. U. S. Dept. Agr. Bul. 1193, 42 p. 1924.

<sup>4</sup> ANDERSON, M. S. THE HEAT OF WETTING OF SOIL COLLOIDS. Jour. Agr. Research 28: 927-935. 1924.

<sup>5</sup> WOOD, R. W., and LOOMIS, A. L. THE PHYSICAL AND BIOLOGICAL EFFECTS OF HIGH-FREQUENCY SOUND WAVES OF GREAT INTENSITY. Phil. Mag. and Jour. Sci. 4: (7). 417-436, illus. 1927.



crystal cut in a certain manner contracts when opposite electrical charges are applied to the opposite faces and expands when the charges are reversed. If the frequency of this alternating high potential is the same as the natural longitudinal frequency of vibration of the piezoelectric crystal, waves of high amplitude are generated, so that considerable sound energy is delivered by the crystal to the medium surrounding it. In this instance the alternating potential, roughly estimated at 25,000 volts, was supplied by a 1,500-watt vacuum tube oscillator tuned to the resonant frequency of the crystal. The crystal was immersed in an oil bath and its energy was transmitted through the oil into an Erlenmeyer flask containing a water suspension of the soil material to be treated.

The oscillator was adapted from a United States Navy transmitter installed in the high-tension laboratory of Fertilizer and Fixed Nitro-

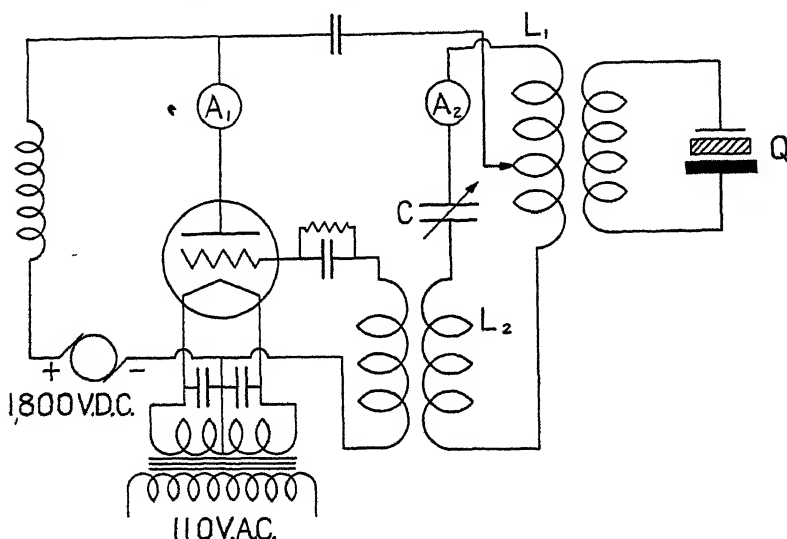


FIGURE 1.—Circuit diagram of the supersonic oscillator, A<sub>1</sub> and A<sub>2</sub>, ammeters; L<sub>1</sub> and L<sub>2</sub>, inductances; C, variable condenser; Q, quartz crystal

gen Investigations of the Bureau of Chemistry and Soils. A diagrammatic sketch of the circuit, which is the Hartley circuit, is shown in Figure 1, and the parts of the apparatus used are shown in Figures 2 and 3. The oscillator employed six 250-watt vacuum tubes in parallel. The filaments were heated by 60-cycle alternating current supplied by a low voltage transformer, and the plate current was supplied by an 1,800-volt direct-current generator. The inductances of the tuned circuit L<sub>1</sub> and L<sub>2</sub> (fig. 1) were made of edgewise wound copper ribbon. The capacitance C of the tuned circuit consisted of a variable air condenser connected in parallel with a fixed one. The circuit driving the crystal Q was inductively coupled to the plate coil of the oscillator L<sub>1</sub> by a single layer solenoid, consisting of about 300 turns of No. 28 double silk-covered copper magnet wire wound on a glass rod form having a diameter of 4 inches. The terminals of this coil were connected through large leads, in order to prevent corona losses, to the electrodes in contact with the quartz crystal.

The lower electrode was made of flat sheet lead about 1 cm. thick, upon which the quartz crystal rested. The upper electrode was made of sheet brass 0.03 mm. thick, and was cut slightly smaller in diameter than the quartz disk upon which it lay. The electrodes and crystal were placed in a small copper steam bath supported by the handles in a larger Pyrex crystallizing dish. (Figs. 2 and 3.) By this arrangement water could be run into the crystallizing dish to cool the contents of the steam bath. The quartz crystal and electrodes were covered to a depth of about 1.5 cm. with a high-grade transformer oil. The piezoelectric crystals used were flat circular disks of crystalline quartz about 8 cm. in diameter and 1 cm. and 0.85 cm. thick, respectively. Their vibration frequencies were, respectively, about 300,000 and 348,000 complete oscillations a second.

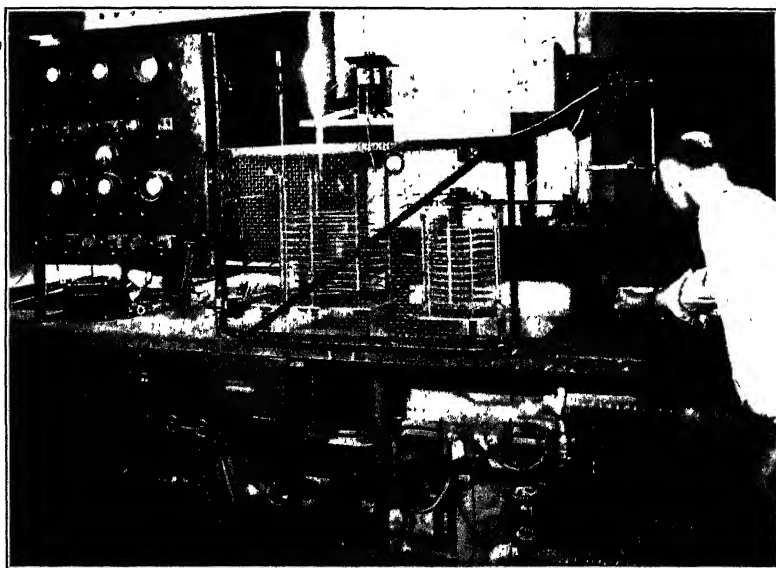


FIGURE 2.—Supersonic oscillator showing the vacuum tube control panel (left), the tuned driver circuit (center), and the radio frequency transformer and bath containing the piezoelectric crystal (right). Beneath the bench is the 1,800-volt motor generator set

When the oscillator was in operation the oil in which the quartz crystal was immersed became vigorously agitated and rose in a mound several centimeters above the crystal, giving the appearance of a fountain from which oil drops were frequently shot out for some distance. The soil material to be supersonically treated was placed in an extra tall Erlenmeyer flask to which water was added and the flask lowered into the mound of agitated oil. The maximum transmission of energy through the bottom of the flask from the oil into the soil suspension occurred when the bottom of the flask was parallel to the crystal face and certain distances away.

Boyle and Lehmann<sup>6</sup> have shown experimentally that Rayleigh's formula for the transmission of a train of plane sound waves

<sup>6</sup> BOYLE, R. W., and LEHMANN, J. F. PASSAGE OF ACOUSTIC WAVES THROUGH MATERIALS. Roy. Soc. Canada, Trans. 21 (pt. 1, sec. 3): 115-125, illus. 1927.

through a partition holds for supersonic waves. According to Rayleigh, the sound transmission is greatest when the thickness of the partition (Pyrex glass in this case) is an integral number of half-wave lengths. At a frequency of 348 kilocycles a half-wave length of sound in Pyrex glass is about 7.5 mm. Since it was not convenient to make an Erlenmeyer flask with its bottom of this thickness, it seemed preferable to approximate zero thickness as closely as possible. Accordingly, a flask was made whose bottom was flat, about 0.7 mm.

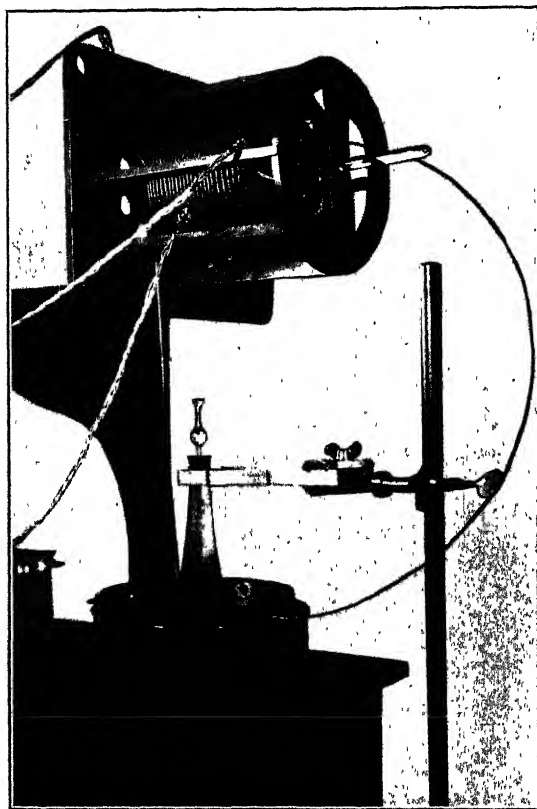


FIGURE 3.—Larger view of the radio frequency transformer and the bath containing the crystal in operation. In the flask may be seen the disturbed soil suspension and, on the inner walls, some of the standing waves

thick, and about 1 cm. smaller in diameter than the quartz crystal. Of much more importance than the thickness of the flask bottom is its position. The flask should be so held that its bottom is parallel to the face of the quartz disk and at such a distance that waves reflected from the glass surface strike the quartz plate in phase with its own vibration. This allows wave trains of large amplitude and high energy to build up in the oil by multiple reflections and eventually to transmit this energy to the material in the Erlenmeyer flask.

A thick plate of high-density material (lead) was used for the lower electrode in order to reflect as much energy as possible up through the crystal. The electrode lying on the upper surface of the crystal was made of the thinnest available metal (brass shim) in order to interfere as little as possible with the upward transmission of energy in the oil. The flask was held by a wooden test-tube clamp attached to a laboratory tripod stand. The tripod base was provided with leveling screws, and the support rod was made of bakelite in order to avoid spark discharges to the hand when adjustment of the leveling screws was necessary.

When subjected to supersonic treatment the suspension in the Erlenmeyer flask appeared to boil violently. Usually a spray of fine drops filled the flask, resembling condensing steam. In the flask shown in Figure 3 may be seen the agitated soil suspension from

which standing waves of liquid gather on the inner walls of the flask. An opening was provided in the stopper to release air and water vapor without losing soil material. The crystal, oil bath, and suspension all heated rather quickly, the crystal having much the highest temperature. The oscillator was seldom run for periods longer than two or three minutes without cooling the oil bath and crystal. The heating of the suspension is due in part to the fact that the viscosity of the liquid increases as the square of the frequency, and in part to the rapid movement of the suspended particles through the liquid and their collisions while in motion. Under the most favorable conditions a suspension in the flask can be heated from room temperature to 100° C. in about two minutes. Water alone heated more slowly. The most rapid temperature rise recorded was that of about 40 c. c. of water containing blue-green algae, whose temperature rose from 18° to 68° in 30 seconds.

### PROCEDURE

Air-dry soil samples of 10 to 30 gm., which had passed through a screen with round holes 2 mm. in diameter, were put into the special Erlenmeyer flask and from 50 to 125 c. c. of distilled water were added. The flask was then clamped in position about 1 to 2 cm. above the upper face of the piezoelectric crystal and its height adjusted by means of tripod leveling screws to give maximum transfer of energy to the contents of the flask. This position was determined either by observing the violence of agitation of the material within the flask or, better, by adjusting for minimum reading of the ammeter,  $A_2$ , in the oscillator circuit.

After supersonically shaking the sample for 2 minutes, the silt and clay in suspension were decanted through a 300-mesh screen into a 500 c. c. centrifuge bottle. Additional decantations were made until the centrifuge bottle was filled. The soil material remaining in the flask was again supersonically shaken, and the silt and clay was poured off until the sands appeared to be free from colloid coatings. With the soils used this usually did not require more than four or five supersonic treatments.

The silt and clay material was centrifuged at 325 gravity until only particles  $1\mu$  or less in diameter remained in suspension. This colloidal suspension was siphoned into a 1-liter assay flask. The water, carrying some material in solution, was removed by a Pasteur-Chamberland suction filter, the colloid being left in the flask. The sediment in the centrifuge bottle was shaken by hand, the bottle was filled with distilled water, again centrifuged, and the suspension siphoned off. When, after repeated centrifugings, the yield of colloid began to decrease, the silt and clay residue was transferred to the Erlenmeyer flask and supersonically shaken. Centrifugation and supersonic treatment were repeated until the yield of colloid decreased to a low value.

In order to secure a comparative basis for the effectiveness of the supersonic method of dispersion, duplicate samples were dispersed by rubbing. These samples were boiled, rubbed with a rubber "policeman," and decanted repeatedly until no further yield of silt and clay could be obtained from the sands. This separation usually required 15 or more 2-minute rubbings, as compared with four or five

shakings by the supersonic method. The colloid was separated in the same manner in both the rubbing and the supersonic methods. Both samples were centrifuged, as far as possible, at the same time, in order to secure the same size separations. When the yield of colloid in the rubbed sample began to decrease, the suspension remaining in the centrifuge bottle after siphoning the colloid was poured off. The sediment was dried to a moisture content near the sticky point. It was then rubbed, kneaded, and worked with a rubber policeman. Further separations by means of centrifuging and rubbing were continued until the yield of colloid fell to a low value.

### EXPERIMENTAL RESULTS

Table 1 gives the results of the separations obtained by the two mechanical methods of dispersion, and also the results of mechanical analyses by the pipette method, of five soil types.

TABLE 1.—*Mechanical analyses of five types of soils, using three different dispersion methods*

Sample No.	Soil type and source	Depth	Dispersion treatment	Percentage composition		
				Sand	Silt	Colloid
3405	Chester loam, Maryland .....	Inches 30-42	Chemical .....	50.5	35.5	14.0
			Rubbing .....	52.0	30.9	17.1
			Supersonic .....	45.3	36.8	18.0
4251	Norfolk sandy loam, Alabama .....	38-49	Chemical .....	67.0	2.8	30.2
			Rubbing .....	68.4	3.7	28.9
			Supersonic .....	66.6	3.1	30.3
4427	Iredell loam, North Carolina .....	10-20	Chemical .....	11.4	28.0	60.6
			Rubbing .....	16.3	18.1	65.6
			Supersonic .....	15.0	20.4	64.6
186	Cecil clay loam, Georgia .....	9-18	Chemical .....	43.8	17.2	39.1
			Rubbing .....	44.8	14.6	40.6
			Supersonic .....	45.7	12.9	41.4
4440	Davidson clay loam, North Carolina .....	9-36	Chemical .....	16.9	29.1	54.0
			Rubbing .....	18.4	23.7	57.9
			Supersonic .....	14.7	25.6	59.7

\* Analyses by the pipette method.

In the mechanical methods of separation the upper limit of colloid size was between  $1\mu$  and  $2\mu$  in diameter. In the pipette method the separation was made at  $2\mu$ . The material listed as silt comprises all particles with diameters between  $50\mu$  and  $2\mu$ , that is, it consisted of all the silt and some coarse clay. In the pipette method used by this bureau<sup>7</sup> the organic matter is removed by hydrogen peroxide. The organic matter was not removed in the rubbing and supersonic methods, but the quantity was small in all samples. In the pipette method, dispersion was obtained by shaking with sodium hydroxide in the Cecil and Davidson samples, and the other samples were dispersed with sodium oxalate. On an average, the supersonic treatment yielded about the same amount of sand as the chemical treatment but less than the rubbing treatment.

Except in the Norfolk sandy loam, both mechanical methods yielded less silt and more colloid than did the pipette method. The samples from the Chester B<sub>2</sub> horizon were rich in flakes of partly

<sup>7</sup> OLMSTEAD, L. B., ALEXANDER, L. T., and MIDDLETON, H. E. A PIPETTE METHOD OF MECHANICAL ANALYSIS OF SOILS BASED ON IMPROVED DISPERSION PROCEDURE. U. S. Dept. of Agr. Tech. Bul. 170, 23 p., illus. 1930.

decomposed mica, which appeared to have been broken down into smaller particles by mechanical treatments.

The Cecil and Davidson soils are lateritic in character, and apparently have many colloid particles cemented into aggregates. Both soils flocculated quickly while being centrifuged. Very small quantities of colloid remained in suspension at the end of 20 minutes in the centrifuge when distilled water with a hydrogen-ion exponent of 6 or less was used to suspend the material in the centrifuge bottles. When just enough ammonia was added to the distilled water supply to bring it to neutrality, the amount of colloid remaining in suspension after centrifuging was abundant. In making the colloid separations of the Cecil and Davidson samples brom-thymol blue, put into the filter flasks, was used as an indicator and assisted in maintaining the suspension at about pH 7. If the material could have been centrifuged quickly in a continuous flow, high-speed centrifuge, it is possible that the colloid could have been separated with the addition of little or no ammonia. With the equipment available it was necessary for the material to remain deflocculated at least 20 minutes. Although ammonia was necessary for temporarily maintaining dispersion, it was not essential for obtaining it. Strictly speaking, these two methods of extraction are not purely mechanical.

The addition of ammonia to the silt and colloid suspensions of the other samples, although not necessary for separation of the colloid, greatly increased the yield and shortened the time required for a separation. For these three soils, the Iredell, the Chester, and the Norfolk, enough ammonia was used to give the water removed in concentrating the colloid a pH value of about 6.4 to 6.8.

The use of a small amount of ammonia as a deflocculent probably did not increase the total yield of colloid, but it did lessen the number of pourings required to make a separation. The number of centrifugings varied from about 30 for the Norfolk to about 90 for the Cecil and 120 for the Davidson when dispersion was obtained by rubbing. About the same degree of separation was obtained with half as many centrifugings when the samples were supersonically shaken. The exact number of operations is not significant because it varies with the amount of rubbing or supersonic treatment, the acidity of the suspension medium, the amount of shaking used in resuspending the sediment in the centrifuge bottles, and the duration of time of centrifuging. Because of the large number of operations involving many transfers of small amounts of material to and from large containers, the methods of separation do not favor high accuracy in the final quantitative results.

Some further difficulty was experienced in making the colloid separation. When the centrifuge was slowing down to a stop the bottles turned from a horizontal to a vertical position, causing a cyclonic motion to the suspension within, which stirred up some of the finest sediment in the bottom of the bottles. Because of this action it is likely that the upper limit of colloid size was nearer  $2\mu$  than the estimated  $1\mu$  in diameter.

In order to determine to what extent the soil particles were abraded by supersonic treatment, mechanical analyses were made of four samples of a Norfolk fine sandy loam subsoil. This soil was chosen because it had a desirable range of particle size distribution, was low

in organic matter, and was easily dispersed. The results are given in Table 2. Samples A and B are untreated duplicates and serve to indicate the accuracy of analysis. Sample C was given supersonic treatments for periods totaling 30 minutes and sample D was similarly treated for 60 minutes. All samples were shaken with the same amount of deflocculent, and analyses were made by the pipette method. The results all check within the accuracy of sampling and of analysis with the possible exception of the clay and colloid fractions. The colloid determination is usually the most accurate, and the silt, which is determined by difference, is the least reliable. It is possible that colloid coatings were more completely removed from the sand and silt fractions and more aggregates of coarse clay were broken down by supersonic treatment, but there is no indication of grinding of the sands. Sample C was shaken in a smaller flask than was D and may possibly have had as drastic treatment in 30 minutes as was given sample D in 60 minutes. The apparatus can be adjusted only to the maximum apparent activity.

TABLE 2.—*Mechanical analysis by pipette method of a Norfolk fine sandy loam subsoil<sup>a</sup> made to determine soil-particle abrasion by supersonic treatment*

Sample	Percentage composition							
	Sand of particle sizes indicated					Silt	Clay	Colloid <sup>b</sup>
	2.0 to 1.0 mm.	1.0 to 0.5 mm.	0.5 to 0.25 mm.	0.25 to 0.1 mm.	0.1 to 0.05 mm.	0.05 to 0.005 mm.	<0.0005 mm.	<0.002 mm.
A <sup>c</sup> .....	0.5	2.6	3.8	15.2	18.9	33.0	26.0	21.7
B <sup>c</sup> .....	.5	2.5	3.7	14.9	19.7	32.3	26.3	21.5
C <sup>d</sup> .....	.5	2.6	3.5	14.9	19.5	32.0	26.9	23.5
D <sup>e</sup> .....	.7	2.6	3.7	14.6	19.7	31.7	27.0	22.3

<sup>a</sup> Analysis by Hubert W. Lakin and Thomas M. Shaw.

<sup>b</sup> Included in the clay.

<sup>c</sup> Untreated duplicate.

<sup>d</sup> Treated supersonically for 30 minutes.

<sup>e</sup> Treated supersonically for 60 minutes.

In the foregoing experiment the entire sample was treated supersonically without making any separations of the finer material. In obtaining the results shown in Table 1 the sands were removed after the first few minutes of treatment and additional supersonic shaking was given to the silt and undispersed clay fractions. The supersonic treatment of the silt fraction probably was not so effective as when larger sand grains were present. As soil particles decrease in size, their momenta correspondingly decrease. On the other hand, the influence of the water films surrounding the particles increases with decreasing size until finally the particles subject to supersonic treatment are no longer able to break through the films and collide with other particles, or strike the walls of the containing vessel. The presence of the sands should aid in the removal of colloidal films from the silt grains and in the breaking apart of fine colloidal aggregates. Just how much of the effectiveness of supersonic treatment is due to actual collision of particles, how much to their large and alternating positive and negative accelerations, and how much to the turbulent flow of the suspension medium, is problematical. The viscosity of

water subjected to periodic compressional waves varies directly as the square of the wave frequency.

It is comparatively easy to cause grinding of sand grains in the rubbing method, and for this reason the rubbing was gently done in an excess of water during the process of separating the sands from the silt and clay. A microscopic examination of the sands revealed some colloid coatings on the rubbed sands but practically none on those supersonically shaken. On the other hand, it seems likely that abrasion caused by rubbing the silt and clay fraction in the centrifuge bottles by a rubber policeman reduced the quantity of silt. When the moisture content of the silt and clay mixture is reduced sufficiently it becomes rather stiff so that a heavy pressure can be applied with the rubber policeman. This method, under favorable conditions, is surprisingly effective in dispersing the colloid fraction.

In making the mechanical separations of the Cecil and Davidson soils, 30-gm. air-dry samples were used, and an attempt was made to fractionate the colloid. These fractions were not based on size separations but on the ease with which the colloid was dispersed. The first and largest fractions were obtained without rubbing or supersonically treating the silt fraction, although the sands were first cleaned by such treatments. When the yield of colloid decreased to a relatively low value, the material separated out was dried, weighed, and labeled "first fraction." The silts were then rubbed and supersonically shaken, respectively, a number of times, until the yield of colloid after treatment was small. The colloid so removed constituted the second fraction. The third and smallest fraction comprised the colloid subsequently removed by more vigorous and prolonged dispersion treatment. The colloid separation even then was not complete. A small and decreasing yield was obtained so long as the dispersion treatments were continued, with no indication of an end point such as occurs in the separation of silt and clay from sand by decantation. The removal of the third fractions to the extent obtained here required as many pourings as and more dispersion treatments than were required for the first and second fractions combined. The total amounts of colloid extracted from the Cecil and Davidson soils are given in Table 1, and the relative amounts of the different fractions are given in Table 3.

TABLE 3.—*Relative amounts of colloids fractionated from samples of Davidson and Cecil clay loams*

Soil type	Percentage of colloids in—					
	Rubbed sample, fraction No.			Supersonically shaken sample, fraction No.		
	1	2	3	1	2	3
Davidson clay loam.....	64	20	16	58	27	15
Cecil clay loam.....	75	22	3	75	20	5

The quantity of some fractions of extracted colloid was too small for making extended physical tests, so that only water-vapor absorption determinations were made. Air-dry samples were exposed in vacuum



desiccators at reduced pressure for five days to an atmosphere in equilibrium with a 3.3 per cent sulphuric acid solution at 35° C. The quantities of water vapor absorbed per gram of material are given in Table 4 for the silt and colloid of the Norfolk and Iredell soils, as well as for the silt and fractionated colloid of the Cecil and Davidson soils.

TABLE 4.—Water vapor absorbed per gram of material over 3.3 per cent sulphuric acid

Soil type	Rubbing treatment				Supersonic treatment			
	Colloid fraction No.			Silt	Colloid fraction No.			Silt
	1	2	3		1	2	3	
Davidson clay loam.....	Gm. 0.306	Gm. 0.313	Gm. 0.238	Gm. 0.044	Gm. 0.295	Gm. 0.315	Gm. 0.237	Gm. 0.043
Cecil clay loam.....	.233	.228	.140	.013	.221	.254	.123	.013
Norfolk sandy loam.....	.272			.037	.273			.016
Iredell loam.....	.289			.024	.289			.024

These determinations were made primarily for the purpose of ascertaining whether the colloid obtained by the two methods had been differentiated by the processes of separation. The first fractions of both the Davidson and the Cecil colloid were removed with just sufficient dispersion treatment to clean the sands of colloidal material. Consequently their water-vapor absorptions by the two dispersion methods should check approximately. It is in the second and third colloid fractions of the Cecil and Davidson soils and in the total colloids of the Norfolk and Iredell soils that the water-vapor absorptions might be expected to show differentiations due to the methods of separation. The data show no such differentiation. Determinations of the water-vapor absorption of some of the separates over 30 per cent sulphuric acid gave as close agreement between fractions obtained by the two methods as between duplicates of the same fraction. The absorptive capacity of the third fraction is considerably lower than that of the first and second. The last small amount of material removed with the most drastic treatment, which constitutes the third fraction, probably contains more broken pieces of cemented aggregates and a relatively smaller amount of fine colloid, and should not be expected to have so high a water-vapor absorption. The term "colloid" as used here was intended to include all soil particles smaller than  $1\mu$ , whether original soil minerals or products of decomposition. Because of the methods of separation the upper size limit, however, is probably nearer  $2\mu$ .

The water vapor absorbed per gram of silt ranged from approximately 5 to 15 per cent of that of the corresponding colloid. Since the specific absorption of pure silt is probably very low the absorptive capacity of the silt fractions should indicate whether colloidal material is still present in those fractions, except for the presence of organic matter. Both colloid and organic matter are known to be present in small amounts. Here again the water-vapor absorption data indicate that the two methods were about equally effective in removing colloid from the silt fractions, although from a qualitative

comparison of the colors of the material and a microscopic examination of the silt grains, the supersonic method appeared to give the better dispersion. Although the sands were rubbed gently in excess water, the silt fractions were rubbed vigorously at such low moisture content as to allow comparatively heavy pressure to be applied to the plastic mass. One may conclude from the data of Table 1 that rubbing caused some grinding of the silt grains. Qualitative observations indicated that if the silts had been rubbed at a higher moisture content, in which condition less pressure is required to work the mass, much less colloid would have been removed, or, at least, more rubbing would have been required. As a consequence of the writer's experience during the prosecution of this work, his respect for the rubbing method has greatly increased, when the operation is carried out with silt and clay materials in a moderately stiff plastic condition.

The advantage of the supersonic over the rubbing method of dispersion lies in the greater ease with which it may be carried out. The number of supersonic shakings is about half the number of rubbing treatments, and the time required is even more favorable to the supersonic method. It takes much longer to dry down the material in a centrifuge bottle to the sticky point and rub it thoroughly than it does to transfer it to an Erlenmeyer flask and shake it supersonically.

A disadvantage of the supersonic method is that considerable expensive equipment is required to build the piezoelectric oscillator. The piezoelectric quartz plates, large enough to furnish sufficient energy, must be cut from large quartz crystals, free from twinning and flaws and capable of withstanding rapid temperature changes and mechanical shock without shattering. Such quartz plates are not plentiful. Another disadvantage is that only small samples, probably not larger than 50 gm., can be effectively treated at one time with crystals of the size used and the power available. When the rubbing is done on large samples the hands may be used instead of a rubber policeman. The fine ridges of the palms of the hands are said to be very effective aids in the dispersion of soil colloids.

Both methods gave as good dispersion as the usual routine method of mechanical analysis which employs chemical aids. Although both rubbing and supersonic shaking are effective in securing dispersion, they can not maintain it. Either a separation must be made before flocculation occurs, which in the Davidson and Cecil soils is only a few seconds, or a chemical deflocculent must be used.

#### SUMMARY

A method of dispersing soil material by means of supersonic waves is described. A soil suspension is shaken by high-frequency, high-intensity sound waves produced by a large piezoelectric quartz crystal which is driven by a 1,500-watt vacuum-tube oscillator. The percentage of colloidal material extracted from soils by the new method is about the same as is secured by a rubbing method and is slightly greater than the percentage obtained from analyses by the pipette method. In neither the supersonic nor the rubbing method of dispersion was the extraction complete. A small and decreasing yield of material of colloidal size was obtained as long as the extraction

processes were continued. The results, however, are much more quickly and easily obtained by the supersonic method.

Determinations of the water vapor absorbed over 3.3 per cent sulphuric acid by the silt and colloid fractions showed no differences between the material extracted by the two methods. The water vapor absorbed per gram by the silt fraction ranged from 5 to 15 per cent of the absorption of the corresponding colloid.

# PREDICTING GAINS IN FEEDER CATTLE AND PIGS<sup>1</sup>

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## INTRODUCTION

Biometrical studies of data from group-feeding experiments with farm animals have generally shown a large amount of variation in the response of different animals in the same lot to what were supposed to be the same conditions of feed, housing, and care. Thus Mitchell and Grindley (8)<sup>4</sup> in an extensive study found coefficients of variability of individual gains to average about 21 per cent for sheep and 17 per cent for swine and steers. With variations as large as these they calculate that it would require 31 sheep per lot or 20 swine or steers per lot to prove (in the statistical sense) the reality of the difference when one lot makes gains 10 per cent greater than those of the other lot. Not only are most experimental lots smaller than these, but most observed differences in gains are also less than 10 per cent, except where the rations to be compared are so very different that the experimenter is fairly certain in advance which is the better.

This high variability in gains is sometimes inferred to be an index of the amount of experimental error attaching to all findings from group-feeding experiments. However, the conclusions drawn from feeding experiments are based not on average gains alone but also on average feed consumption, average quality of the final product, and various ratios or other combinations of these three kinds of primary data. The most appropriate statistical procedure for testing the validity of conclusions drawn from group-feeding experiments seems to be Student's method (1) applied to the conclusions drawn from several repetitions of an experiment.<sup>5</sup> This method can not be used until the experiment has been repeated at least once, and more and more repetitions will be required to prove the reality of smaller and smaller differences between the values of the rations tested. This method makes no direct use of individual gains. It is often desirable to estimate the significance of observed differences, at least in a tentative way, before an experiment has been repeated often enough to permit Student's method to show the full reliability of the conclusions. Variation in gains is the most accessible basis for such estimates, although much could be accomplished by also taking into account variation in quality of the final product as revealed by individual slaughter or carcass grades or appraisals. Variation in feed consumption can not of course be known in group-feeding experiments.

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<sup>2</sup> The writer is greatly indebted to the following members of the college, station, or extension staffs of the Agricultural and Mechanical College of Texas for making the estimates on which this report is based: G. W. Barnes, R. E. Dickson, E. R. Eudaly, W. E. Flint, C. B. Godbey, J. A. Gorman, Fred Hale, W. R. Horlacher, J. M. Jones, J. H. Knox, A. K. Mackey, W. L. Stangel, D. W. Williams, and R. H. Williams.

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<sup>4</sup> Reference is made by number (italic) to Literature Cited, p. 881.

<sup>5</sup> For an example of this method applied to feeding data, see Jones and Lush (3).

When the variation in gain is to be used as an index of the amount of experimental error it should be kept in mind that at least three factors other than errors of sampling may cause the calculated standard deviation of gains within a lot to be relatively larger than the real experimental error with which the differences should be compared. The first is the fact that initial and final weights are not exactly correct because of differences in "fill," undetected minor differences in health at weighing time, etc. Such errors cause the apparent standard deviation to be larger than the standard deviation of the true gains would be if these could be obtained free from such errors. The observed standard deviation can be corrected (10) roughly for this source of error. When such corrections are made it becomes apparent that this source of error usually accounts for only a small part of the observed standard deviation,<sup>6</sup> although it will assume considerable importance when the feeding periods are short and when initial and final weights are taken on only one day. It is probably of more importance with ruminants than with swine.

The second factor tending to magnify observed standard deviations of gains is inequality in feed consumption within a lot. To the extent that the steer or pig which made the high gains in a lot did so directly because it ate more feed than its per capita share, the standard deviation of the observed gains will be larger than it would have been if each steer or pig had eaten exactly the average ration for its lot. The importance of this depends primarily upon how high the correlation is between individual feed consumption and individual gain in the usual feeding experiment. The principles of nutrition lead one to expect that such correlations will usually be high, although doubtless quite variable from one experiment to another. It seems likely that this factor is of considerable importance in making the apparent probable error of feeding experiments higher than the error which should be used when gains are considered in relation to feed consumption, as is nearly always the case.

The third factor tending to magnify the observed standard deviation of gains is the fact that the experimental animals are not divided into lots by a random method but are more or less carefully balanced with respect to weight, breed, sex, age, and their conformity to the standard of excellence which the man in charge of the experiment has in mind. Sometimes even body measurements are taken into consideration in this balancing process, and, in the case of pigs, each litter is distributed evenly among the lots. Not all these factors receive equal attention. Weight is the primary basis of allotment in most cases. After the allotment by weights has been completed, the man in charge of the experiment usually studies each group and switches a pair of animals here and there between two lots until he is satisfied that the lots are as nearly equal as possible in market desirability, degree of finish, quality, and ability to gain weight. This

<sup>6</sup> For example, in one Texas feeding experiment the apparent standard deviation of total gains (based on 51 degrees of freedom) was 45.4 pounds for steers fed for 114 days and weighed three times at the beginning and end of the experiment. If a standard deviation of 10 pounds be assumed for the accuracy of single weights, the true standard deviation will be 44.7 according to Shewhart's formula:  $(45.4)^2 = 2 \times \frac{(10)^2}{3} + (44.7)^2$ . It may be that 10 pounds is too small a figure for the error of single weights. It corresponds closely to the findings in studies of the day-to-day variation in live weight but would not include fills or shrinks extending over several consecutive days, as might result from a digestive disturbance or some other variation in health which, while temporary, would normally extend over more than one day. See Maymone and Sircana (7).

balancing according to judgment is frequently done in the case of steers, but less often with swine, where litter and age distribution assume much importance. Very little of this balancing according to judgment is done in feeding experiments with sheep.

The purpose of all this balancing is to make sure so far as possible that a high-gaining animal in one lot will be matched by an equally high-gaining animal in every other lot, and that a low-gaining animal, or one undesirable on the market, if present in one lot will be matched with an animal as nearly like it as possible in every other lot. If this procedure is successful, then the standard deviation of gains within lots will be kept larger than it would be if the animals were divided into lots purely at random. The importance of this balancing will depend upon the extent to which it actually achieves its purpose. This is the subject of the studies reported herein.

The problem may be stated in the form of three questions: (1) To what extent are differences in feed-lot gains and in market desirability correlated with differences in initial weight? (2) To what extent can the animal husbandman in charge of an experiment foretell which individuals will make the better and which the poorer gains? (3) To what extent can he predict the difference in market desirability which will exist between the animals when they are fattened?

#### NATURE AND SOURCE OF DATA

The animals used were steers that had been fattened during three years at substation No. 7 of the Texas Agricultural Experiment Station at Spur, and two years at College Station, and pigs from three feeding experiments carried on in different years at substation No. 10 at College Station.

The feeding of the pigs and steers was determined by the requirements of the projects in which they were used. After the animals had been divided into lots in accordance with those projects, the animal husbandman in charge and several of his associates rated each animal according to the amount of gain which he believed that animal would make relative to the average of its lot or relative to the animal which he believed would make the best gains in that lot. In three of the steer experiments an estimate was also made of what each animal would be worth per pound of live weight when the fattening was over. All the estimators were men trained in animal husbandry, and they occupied positions requiring constant contact with matters relating to animal husbandry. Several of them regularly taught classes in stock judging, and some have, from time to time, judged at State and prominent regional fairs.

In the second steer experiment each animal was rated in terms of the average of his lot. That is, a steer which was expected to gain 6 per cent more than the average of his lot was rated at 106 and one which was expected to gain 10 per cent less than the average of his lot was rated at 90. This, however, seemed to most of the men rather artificial and contrary to their natural method of judging. In all other experiments each man first picked out the individual which he thought would make the largest gain in the lot and this animal was given a rating of 100. Then he picked out the individual which he thought would make the second largest gain and rated it 98 or 95, or whatever percentage of the first one's gain he thought the

second would make. This was continued until every animal in the lot was rated. Then the next lot was rated with reference to the animal in it which was expected to make the largest gains. This was continued until all lots in the experiment had been rated. Some men worked rapidly and rated as many as 40 animals in little more than an hour. Others took more time and some made a tentative rating one day and returned to revise it a day or two later. Except in the first pig experiment, each man was requested to work independently of the others and to take as much time as he felt would be necessary to make the best possible estimates. In the first pig experiment the three men acted as a committee, consulted freely and turned in only a single set of estimates. All estimates were given to the writer within two weeks after the beginning of each experiment and before any intermediate weights were taken.

The experiments with pigs were ration tests made under the direction of Fred Hale. All rations were reasonably good. All gains were likewise good, the pigs averaging more than 1.5 pounds per head per day in all three experiments. The steers of the first and second experiments are described in Bulletin No. 385 (4) of the Texas station (see especially Table 1) as the 1924, 1925, 1926, and SMS steers. The steers of the third experiment were similar in breeding to those described in Bulletin 385 as 1924, 1925, and 1926 steers, but were born and fed at later dates. The steers of the fourth and fifth experiments were fed under the direction of J. H. Knox in a test involving a comparison of various market grades of feeder steers. All steers were given good rations and all made good gains, only one lot failing to gain at least 2 pounds per head per day.

#### METHODS OF CALCULATION

Correlation coefficients were calculated by the usual product-moment method for each lot of pigs or steers separately, observed gain, initial weight, and each estimate of gain being used as the variables. All possible correlations between these variables were calculated, thus making from as few as 3 correlation coefficients for each lot in the first steer experiment, where only 1 man took part, to as many as 78 correlation coefficients for each lot in the fourth steer experiment, where 11 men took part. The calculations for each lot were made separately in order not to include in the coefficients any effects of differences in rations or in breeding of the different lots. Since the numbers in each lot were small, the observed coefficients tended to be distinctly biased in the direction of largeness whether plus or minus. A correction for this was made according to the method suggested by Fisher (2, p. 171-172) whereby the quantity

$\frac{r}{2(n-1)}$  is subtracted from the  $Z$  value corresponding to the observed  $r$ .

The correlation coefficients for the same two variables for all the different lots in the same experiment were averaged together according to Fisher's method to obtain an average correlation coefficient for the different lots in each experiment. The calculation of this average is illustrated in Table 1.

TABLE 1.—Illustration of method used in averaging correlation coefficients<sup>a</sup>

[Correlation between initial weight and gain, third steer experiment]

Designation of lot	Number of steers in lot	Degrees of freedom <sup>a</sup> in Z	Observed r	Corresponding Z value	Correction for bias of small numbers	Corrected Z value	Corrected Z value multiplied by degrees of freedom
1927 Herefords .....	4	1	+0.929	+1.6510	+0.1548	1.4962	1.4962
1927 Brahmans .....	13	10	+0.570	+0.6475	+0.0238	.6237	6.2670
1927 Back crosses .....	9	6	+0.455	+0.4910	+0.0284	.4626	2.7756
1928 Herefords .....	6	3	-.092	-.0923	-.0092	-.0831	-.2493
1928 Brahmans .....	11	8	+0.123	+0.1236	+0.0062	.1174	.9392
1928 Back crosses .....	14	11	+0.323	+0.3350	+0.0124	.3226	3.5486
Total .....	57	39	.....	.....	.....	.....	14.7473

Average Z=0.3781. Corresponding average  $r=+0.361$ .

<sup>a</sup> "Degrees of freedom" is a technical statistical term used to designate the number of items really free to vary in the data from which the statistic in question was calculated. In general the number of degrees of freedom is only slightly less than the total number of items but may be very distinctly less for averages based upon a large number of subgroups each calculated separately, as was the case in this study.

This method of averaging seems to the writer better suited to these data than any other method yet suggested. It is not entirely adequate to the problem of averaging all the intercorrelations of several variables, but adequate methods for that have not yet been developed. A suggestion of the extent of error in the methods employed may be gained from the fact that, when these average  $r$ 's are used, the squared multiple correlations between final weight as the dependent variable and initial weight and gain as the independent variables (which, of course, should equal 1.000, all primary correlations being carried to the third place) are found to be as follows:

First pig experiment .....	0.950
Second pig experiment .....	.939
Third pig experiment .....	.999
First steer experiment .....	.978
Second steer experiment .....	1.006
Third steer experiment .....	1.036
Fourth steer experiment .....	1.060

These discrepancies are slight but may be large enough to cast some doubt upon the reality of small differences which the further analysis of these average correlations might seem to show. Consequently, the correlation coefficients are also given as actually calculated without correction for bias due to small numbers. Where this would be too cumbersome and space-consuming the distributions of the correlation coefficients are presented.

## RESULTS

### FIRST EXPERIMENT WITH PIGS

In the first experiment there were five lots of eight pigs each, which were fed for 70 days and made an average daily gain of 1.55 pounds from an average initial weight of 80 pounds. The average intralot standard deviation (35 degrees of freedom) of daily gains was 0.207, thus giving a coefficient of variability of 13.3 per cent. The three men who acted as a committee freely discussed each pig and agreed upon a single set of estimates. This procedure did not seem entirely satisfactory to any of the men and in all later experiments each man



worked independently. The results are shown in Table 2. The symbols are:  $G$ =gain,  $J$ =judgment or estimate of gain,  $I$ =initial weight.

TABLE 2.—Correlation coefficients from first feeding experiment with pigs

Symbol <sup>a</sup>	Correlation coefficients for pigs in—					Average by Fisher's method
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	
$r_{GI}$ .....	+0.16	-0.18	-0.28	+0.53	-0.03	+0.05
$r_{GI}$ .....	+0.01	-0.31	-0.32	+0.50	-0.18	-0.05
$r_{JI}$ .....	+0.96	+0.95	+0.96	+0.98	+0.92	+0.95
$r_{GIJ}$ .....	+0.52	+0.38	+0.11	+0.26	+0.34	+0.32
$r_{GIJ}$ .....	-0.50	-0.45	-0.20	-0.16	-0.38	-0.32
$R_{GIJ}$ .....						.33

<sup>a</sup> See text for explanation.

Here  $r_{JI}$  is uniformly high and  $r_{GIJ}$  is slightly larger when positive and smaller when negative than  $r_{GI}$ . This seems to mean that the men in making their estimates were influenced greatly (far more than they suspected) by the differences in initial size of the pigs. In this experiment (unlike most of the later ones)  $r_{GI}$  tends to be slightly negative and, because of the very strong association of  $I$  and  $J$ ,  $r_{GIJ}$  is brought down to values nearly as low as  $r_{GI}$ . This is shown also by the partial correlation coefficients, among which those between gain and judgment, initial weight being constant, are uniformly positive and are of considerable size, while those between gain and initial weight, judgment being constant, are as uniformly negative and are also of considerable size. Thus it appears that in this experiment the things associated with initial weight but regarded as unimportant by the judges were negatively correlated with gains. On the other hand, the things which the judges considered but which were not reflected in initial weight were positively associated with gain. So closely were judgment and initial weight associated that the primary correlation coefficients between either of them and gain are not far from zero. Balancing the lots according to initial weight alone or according to judgment alone would have had practically no effect on the standard deviation of gains. And yet if the pigs had been exactly alike both in initial weight and in the opinion of the judges, the variation in the gains would have been about 5 per cent less than it actually was.

#### SECOND EXPERIMENT WITH PIGS

Six lots of 10 pigs each were fed for 70 days and made an average daily gain per head of 1.73 pounds from an average initial weight of 90 pounds. The average intralot standard deviation (54 degrees of freedom) of the individual daily gains was 0.169 pound, thus giving an apparent coefficient of variability of 9.8 per cent—a quite uniform group of gains. Three men participated in this experiment, each making his estimates without consulting the others although all were made at the same hour, a day or two after the pigs had been divided into lots for experimental purposes. The results are shown in Table 3. The symbols  $A$ ,  $B$ , and  $C$  represent the estimates made by the three men and  $I$  and  $G$  represent initial weight and gain, as before.

TABLE 3.—*Correlation coefficients from second feeding experiment with pigs*

Symbol <sup>a</sup>	Correlation coefficient for pigs in—						Average by Fisher's method
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6	
<i>r</i> <sub>GA</sub> -----	0.79	0.67	0.48	0.88	0.53	0.43	0.65
<i>r</i> <sub>GB</sub> -----	.90	.68	.66	.73	.21	.73	.68
<i>r</i> <sub>GC</sub> -----	.58	.69	.33	.78	.44	.47	.55
<i>r</i> <sub>GI</sub> -----	.89	.70	.35	.68	.40	.58	.62
<i>r</i> <sub>IA</sub> -----	.83	.90	.94	.85	.92	.57	.85
<i>r</i> <sub>IB</sub> -----	.90	.90	.80	.94	.86	.81	.87
<i>r</i> <sub>IC</sub> -----	.52	.81	.86	.88	.94	.92	.85
<i>r</i> <sub>AB</sub> -----	.76	.88	.94	.88	.85	.52	.83
<i>r</i> <sub>AC</sub> -----	.69	.97	.89	.87	.97	.59	.88
<i>r</i> <sub>BC</sub> -----	.69	.81	.83	.91	.85	.70	.80

<sup>a</sup> See text for explanation.

In this experiment all correlations are positive and nearly all are large. The correlations between judgments and initial weight are prevailingly high. The different men agreed quite closely with each other. The correlations between judgments and gain are distinctly lower than those between the different judgments or between the judgments and initial weight. Initial weight and gain are correlated almost as closely as the judgments and gain are. The partials between initial weight and gain, one judgment being constant, and the partials between each judgment and gain, initial weight being constant, are much lower than the corresponding primary coefficients. This shows that much of the success which the men had in estimating the gains of the individual pigs came from the attention which they paid to things already largely reflected in initial weight.

The multiple correlation coefficient between gain as the dependent variable and initial weight and the three judgments as the independent variables is 0.707, which indicates that about half ( $R^2$ ) of all the causes of variation in the gains in this experiment were seen by one or more of the judges or were reflected in initial weight.

The multiple correlation coefficient is large enough to be of undoubted statistical significance, but when any one of the four independents is made constant the resulting partials become so small as to raise the question whether the evidence is sufficient to prove their reality. There seems to be no escape from the conclusion that each of the independent variables was positively associated with gain, but that most of this association was shared jointly by all four independent variables. The differences between judges are far below the level of statistical significance.

#### THIRD EXPERIMENT WITH PIGS

At the beginning of this experiment there were 9 lots of pigs each containing 10 animals, but 5 of the pigs became unthrifty and were removed before the end of the experiment. Consequently 5 lots of 10 pigs each, 3 lots of 9 pigs each, and 1 lot of 8 pigs finished the experiment. The pigs were fed for 100 days, and made an average daily gain of 1.60 pounds from an average initial weight of 51 pounds. The standard deviation of the average daily gains of the individual pigs within each lot (76 degrees of freedom) was 0.194 pound, thus giving a coefficient of variation of 12.1 per cent—a more uniform

group of gains than the 17 per cent average found by Mitchell and Grindley (8).

Four men participated in this experiment, but there was much confusion in the pens because of an unfamiliar method of identifying the pigs. Not being entirely satisfied that they had done their best, three of the men went back three days later when the pigs were adequately marked and again estimated the individual gains without referring to their previous estimates. The judges felt more confidence in their second set of estimates, although it may be remarked parenthetically that the data when analyzed did not justify much greater confidence.

TABLE 4.—Correlation coefficients from third feeding experiment with pigs

Symbols *	Correlation coefficients, for pigs in—									Average by Fisher's method
	Lot 1, 9 pigs	Lot 2, 10 pigs	Lot 3, 10 pigs	Lot 4, 8 pigs	Lot 5, 10 pigs	Lot 6, 9 pigs	Lot 7, 9 pigs	Lot 8, 10 pigs	Lot 9, 10 pigs	
<i>r</i> <sub>A1G</sub> .....	0.37 <sup>a</sup>	0.40	0.61	0.72	0.49	0.43	0.73	0.84	0.11	0.59
<i>r</i> <sub>B1G</sub> .....	.81	.53	.53	.81	.64	.57	.67	.88	.28	.64
<i>r</i> <sub>C1G</sub> .....	.69	.31	.51	.79	.60	.56	.65	.86	.28	.59
<i>r</i> <sub>D1G</sub> .....	.80	.42	.71	.68	.43	.78	.73	.77	.37	.63
<i>r</i> <sub>A2G</sub> .....	.85	.33	.53	.87	.50	.60	.84	.87	.47	.67
<i>r</i> <sub>B2G</sub> .....	.78	.40	.51	.83	.42	.69	.72	.89	.42	.64
<i>r</i> <sub>C2G</sub> .....	.91	.37	.65	.86	.54	.40	.73	.92	.39	.68
<i>r</i> <sub>D2G</sub> .....	.88	.35	.60	.86	.36	.65	.70	.83	.24	.62
<i>r</i> <sub>A1I</sub> .....	.91	.84	.89	.89	.81	.78	.93	.93	.88	.87
<i>r</i> <sub>B1I</sub> .....	.84	.93	.94	.77	.84	.84	.83	.80	.95	.87
<i>r</i> <sub>C1I</sub> .....	.73	.85	.96	.80	.86	.96	.97	.99	.96	.93
<i>r</i> <sub>D1I</sub> .....	.79	.83	.85	.92	.88	.87	.92	.91	.98	.89
<i>r</i> <sub>A2I</sub> .....	.81	.98	.98	.93	.92	.92	.89	.93	.95	.94
<i>r</i> <sub>B2I</sub> .....	.87	.96	.95	.84	.85	.88	.96	.91	.92	.91
<i>r</i> <sub>C2I</sub> .....	.94	.95	.97	.94	.91	.86	.96	.93	.97	.94
<i>r</i> <sub>A1A2</sub> .....	.94	.84	.85	.67	.91	.71	.91	.96	.85	.87
<i>r</i> <sub>B1A2</sub> .....	.94	.98	.95	.79	.83	.87	.88	.97	.88	.91
<i>r</i> <sub>C1C2</sub> .....	.78	.90	.92	.81	.91	.91	.96	.91	.94	.90

\* See text for explanation.

Both the first and second sets of estimates were used in the calculations, thus making eight independent variables (the seven judgments and initial weight) and one dependent variable (gain). The results are shown in Table 4. The letters *A*, *B*, *C*, and *D* represent the judgments, the subscript numerals indicating whether the first or the second set of estimates is meant. *I* and *G* indicate initial weight and gain, respectively. The averages by Fisher's method are each based upon 58 degrees of freedom, i.e., they are statistically equivalent to a simple correlation calculated upon a population of 61.

Besides the correlations shown, there were 162 other correlations between the estimates of one judge and those of the other judges. The distribution of these was as follows:

Size of coefficient		Number of coefficients found	
0.45-0.50.....	2	0.75-0.80.....	10
0.50-0.55.....	1	0.80-0.85.....	21
0.55-0.60.....	4	0.85-0.90.....	37
0.60-0.65.....	3	0.90-0.95.....	46
0.65-0.70.....	2	0.95-1.00.....	27
0.70-0.75.....	9		

The average of all these 162 coefficients according to Fisher's method was +0.885.

The correlation coefficients calculated in this experiment may be divided into four groups: (1) Those involving gain, (2) those between initial weight and the seven judgments, (3) those between the first and second judgments of the same men, and (4) those between the judgment of one man and the judgments of the other three. It is evident merely from inspection (and consideration of the averages confirms this) that the correlations of the last three groups were of practically the same magnitude, while those involving gain were distinctly smaller, although still large enough to be of undoubted statistical significance. All of the 324 simple correlation coefficients were positive and only one fell below +0.24.

The fact that the correlations between a man's first judgment and his second judgment were no larger than the correlations between his judgment and the other men's judgments points either to a very considerable degree of uncertainty in his mind when he made the estimates or to very considerable changes in the appearance of the pigs in the three days that intervened between first and second estimates, probably the former.

As in the second experiment, when initial weight is made constant much of the correlation between judgment and gain disappears. It is likewise true that if any one of the seven judgments is made constant much of the correlation between initial weight and gain disappears. In other words, the men were paying attention to things which actually were associated with the gaining ability of the pigs but which were already in large part reflected in differences in initial weight. The multiple correlation coefficient between all eight independent variables and gain was 0.702, thus indicating that almost half ( $R^2$ ) of the causes of differences in gains were foreseen by at least one judge, or were associated with differences in initial weight. When initial weight is left out of consideration the coefficient of multiple correlation falls only from 0.702 to 0.698. This is to be interpreted as showing that practically all of the association between initial weight and gain rested on things also seen by one or more of the men. Likewise if the three estimates which were made on the second occasion are omitted altogether, the multiple correlation coefficient with gain falls only from 0.702 to 0.664. This seems to mean that while the second day's estimates actually were a bit more accurate than the first, yet nearly everything of importance which the men saw on the second occasion was also seen by them on the first occasion.

Whether there were any real differences in the ability of the different judges to predict gains remains doubtful. The differences in some of the first-order partial correlation coefficients seem large enough to border on statistical significance and yet certain facts are opposed to this interpretation. For example, the greatest difference found when initial weight was made constant was not a difference between the estimates of two judges but a difference between the two estimates of one judge. As a group the judges seem rather certainly to have seen a few things which were associated with gain but which were not reflected in initial weight, but the total importance of these things is small compared with that of the things seen by the judges and also reflected in initial weight.

## FIRST EXPERIMENT WITH STEERS

Six lots of steers were fed for 112 days and made an average daily gain of 2.56 pounds, with an average intralot standard deviation (52 degrees of freedom) of 0.271 pounds in the individual average daily gains. The coefficient of variation was 10.6 per cent, which is distinctly less than the average found by Mitchell and Grindley (8). Three of the lots were calves about 8 to 9 months old when feeding began and the other three were one year older. All had been bred and raised at the Texas station. They are described in more detail in Texas Agricultural Experiment Station bulletins 385 (4) and 409 (6) under the designation 1924 and 1925 calves. The results of the experiment are shown in Table 5. *J* represents the estimates; *I*, initial weight; and *G*, gain. The averages shown in the last column are each based upon 40 degrees of freedom—i. e., they are statistically equal to simple correlations calculated upon a group of 43 steers. On populations of this size correlations need to be larger than 0.30 before the probability of their being accidental variations will fall below 0.05, and larger than 0.39 before that probability will fall below 0.01. The estimates were much influenced by factors associated with large initial weight. The other parts of the ideal which the judge applied to these steers were neutral or slightly wrong. The multiple correlation between gain as the dependent variable and initial weight and estimated gain was 0.297, thus indicating that less than 9 per cent ( $R^2$ ) of the causes of the variations in gain in this experiment were either correlated with initial weight or were foreseen by the man in charge.

TABLE 5.—Correlation coefficients from first feeding experiment with steers

Symbol <sup>a</sup>	Correlation coefficients for—						Average by Fisher's method
	Steers born in 1924			Steers born in 1925			
	Here- fords, 10 head	First-cross Brahmans, 14 head	Back crosses 4 head	Here- fords, 10 head	First-cross Brahmans, 14 head	Back crosses 4 head	
<i>r<sub>JG</sub></i> .....	-0.36	+0.05	-0.58	+0.45	-0.36	+0.68	-0.03
<i>d<sub>G</sub></i> .....	+0.05	-0.10	+0.69	+0.58	+0.18	+0.47	+0.19
<i>r<sub>IJ</sub></i> .....	+0.66	+0.80	-0.32	+0.88	+0.46	+0.86	+0.70

<sup>a</sup> See text for explanation.

## SECOND EXPERIMENT WITH STEERS

Six lots of steers were fed for 111 days in the winter of 1926-27. They are the last six lots of steers described in Texas Agricultural Experiment Station Bulletin 385 (4). One 1926 back-cross steer used in that study was omitted from this one, because, the slaughter data on him were incomplete and he could not be used in the estimates of final value. The gains were reasonably good, averaging 2.05 pounds per head per day for the entire group of 63 steers. The average intralot standard deviation (57 degrees of freedom) of the individual average daily gains was 0.223 pound, thus giving a coefficient of variation of 12.0 per cent, which is distinctly less than the average of about 17

per cent found by Mitchell and Grindley (8). Four men took part in the estimates in this experiment. Not only were the gains estimated as in the other experiments but a new feature was added by each man estimating independently what the individual steers would be worth per pound at the close of the experiment. Each judge studied the lot until he was sure that he had a good idea of the differences between the steers. He then rated the future worth of each steer as a certain percentage more or less than the average worth of that lot. (In later experiments it was found more satisfactory to rate the best steer at 100 and to work down from that figure.) The dressed carcasses were appraised by three or more meat salesmen who were unacquainted with the breeding, feeding, or previous history of the individual animals. This average appraised price for the dressed meat from each steer was multiplied by the dressed weight of that carcass and was then divided by the live weight of the steer at the close of the feeding experiment.

Thus there were taken into account both the market desirability of the meat and the differences in yield. The figure thus obtained for the final value of each steer is of course not absolutely accurate<sup>7</sup> but it seemed the most accurate and least subjective standard available.

The results of this experiment are shown in Table 6. *A, B, C*, and *D* represent the estimates of gains, while *a, b, c*, and *d* represent the estimates of the corresponding men on market desirability of the finished steers. *I* is initial weight, *G* is gain, *P* is appraised price per pound of the dressed meat, and *V* is the value of the meat per pound of live weight.

The averages obtained by Fisher's method rest on 45 degrees of freedom; that is, they are based on as large a population as simple correlations calculated upon groups of 48. They need to be larger than 0.28 before the probability of their being chance variations will fall below 0.05 and they should be larger than 0.37 before that probability will fall below 0.01. Thus the correlations involving *G* are probably significant even when considered singly, and certainly so when it is noted that all five are of the same sign and of about the same magnitude. Correlations involving *P* are a little larger than those involving *G*, and those involving *V* are slightly larger than those involving *P*, although neither of these sets of differences is large enough or consistent enough to be unmistakably significant.

Again, as in the second and third pig experiments, there is a high correlation between the different estimates of gain, and a high correlation between initial weight and the estimates of gain. The multiple correlation of *G* as dependent variable with the four judgments and initial weight as independent variables is 0.428, and when initial weight is omitted this figure falls only to 0.421. Eighteen per cent of the causes of variation in the gains of these steers were foreseen by the four men or were reflected in initial weight. While this is perhaps a significant accomplishment on the part of the judges, yet the 82 per cent of the causes of variation unseen by them and not reflected in initial weight shows by vivid contrast the small extent to which the problem of estimating gains was solved.

<sup>7</sup> Its accuracy has been studied in some detail and the conclusions published by Lush, Black and Sample (6).

TABLE 6.—Correlation coefficients from second feeding experiment with steers

Symbol *	Correlation coefficient for—						Average by Fisher's method
	Steers born in 1926			S. M. S. steers			
	Here- fords, 13 head	First- cross Brah- mans, 17 head	Back crosses, 4 head	Fed ground milo heads and whole bundles, 10 head	Fed ground milo heads and chopped bundles, 10 head	Fed whole milo heads and whole bundles, 9 head	
r <sub>AG</sub>	+0.52	+0.47	-0.70	+0.57	-0.08	+0.46	+0.39
r <sub>BG</sub>	+0.55	+0.51	-0.72	+0.21	-0.29	+0.43	+0.32
r <sub>CG</sub>	+0.34	+0.43	-0.32	+0.44	-0.12	+0.09	+0.27
r <sub>DG</sub>	+0.24	+0.46	-0.34	+0.29	-0.15	+0.72	+0.32
r <sub>IG</sub>	+0.60	+0.49	+0.18	+0.34	+0.40	+0.52	+0.36
r <sub>AI</sub>	+0.94	+0.96	+0.66	+0.81	+0.30	+0.91	+0.88
r <sub>BI</sub>	+0.90	+0.95	+0.42	+0.10	-0.14	+0.55	+0.74
r <sub>CI</sub>	+0.85	+0.98	+0.80	+0.90	+0.78	+0.64	+0.90
r <sub>DI</sub>	+0.79	+0.81	+0.17	+0.86	+0.90	+0.92	+0.83
r <sub>AB</sub>	+0.87	+0.92	+0.95	+0.20	+0.18	+0.35	+0.72
r <sub>AC</sub>	+0.82	+0.96	+0.80	+0.88	+0.34	+0.66	+0.84
r <sub>AD</sub>	+0.69	+0.74	+0.52	+0.78	+0.49	+0.93	+0.73
r <sub>BC</sub>	+0.83	+0.93	+0.58	+0.33	-0.55	+0.17	+0.64
r <sub>BD</sub>	+0.65	+0.81	+0.75	+0.09	-0.28	+0.46	+0.61
r <sub>CD</sub>	+0.72	+0.80	-0.01	+0.85	+0.82	+0.52	+0.74
r <sub>AP</sub>	+0.38	+0.63	+0.83	+0.71	+0.07	+0.45	+0.49
r <sub>BP</sub>	+0.37	+0.43	+0.93	+0.27	-0.03	+0.15	+0.30
r <sub>CP</sub>	+0.56	+0.16	+0.96	+0.42	+0.61	+0.16	+0.39
r <sub>DP</sub>	+0.51	+0.40	-0.52	+0.29	+0.19	+0.46	+0.36
r <sub>IP</sub>	+0.45	+0.54	+0.66	+0.42	+0.10	+0.38	+0.41
r <sub>AV</sub>	+0.46	+0.68	+0.93	+0.56	+0.04	+0.33	+0.48
r <sub>BV</sub>	+0.47	+0.46	+0.71	+0.36	-0.00	+0.22	+0.34
r <sub>CV</sub>	+0.56	+0.25	+0.72	+0.19	+0.66	+0.44	+0.41
r <sub>DV</sub>	+0.66	+0.34	-0.45	+0.43	+0.45	+0.42	+0.43
r <sub>IV</sub>	+0.57	+0.61	+0.92	+0.52	+0.29	+0.47	+0.52
r <sub>AI</sub>	+0.67	+0.80	+0.91	+0.17	+0.50	+0.16	+0.59
r <sub>BI</sub>	+0.78	+0.73	+0.39	-0.28	+0.34	+0.00	+0.48
r <sub>CI</sub>	+0.67	+0.72	+0.41	-0.25	+0.59	-0.20	+0.46
r <sub>DI</sub>	+0.71	+0.78	-0.44	+0.65	+0.80	+0.78	+0.72
r <sub>AB</sub>	+0.78	+0.74	+0.62	+0.75	+0.75	+0.74	+0.72
r <sub>AC</sub>	+0.74	+0.43	+0.65	+0.51	+0.38	+0.72	+0.55
r <sub>AD</sub>	+0.49	+0.73	-0.74	+0.44	+0.50	+0.57	+0.54
r <sub>BC</sub>	+0.75	+0.50	+0.98	+0.45	+0.44	+0.57	+0.57
r <sub>BD</sub>	+0.54	+0.74	-0.20	+0.25	+0.33	+0.28	+0.40
r <sub>CD</sub>	+0.50	+0.29	-0.45	+0.04	+0.63	+0.13	+0.32

\* See text for explanation.

Much of what was seen by each man was seen by the others and was also reflected in initial weight. This is shown by the small size of the partial correlation coefficients when initial weight or one of the judgments is made constant. The coefficient of multiple correlation between *V* as the dependent variable and *a*, *b*, *c*, and *d* as independent variables is 0.559, indicating that 31 per cent of the causes of the differences in final value were foreseen by one or more of the four men.

The fact that the estimates of *V* (value of meat per pound of live weight) are slightly more accurate than the estimates of *P* (appraised price of dressed meat) seems to indicate that vagaries of dressing percentage were not the cause of failure to estimate *V* more closely. *V* is merely *P* multiplied by individual dressing percentage. The data indicate (although with doubtful statistical significance) that the men had some success in estimating even so far in advance which steers would be paunchy and "wasty"—a point upon which they had many misgivings when making the estimates.

The men paid less attention to initial weight when they were estimating final values than when they were estimating gains. Also, they agreed with each other less closely when estimating final value than when estimating gain.

### THIRD EXPERIMENT WITH STEERS

Six lots of steers totaling 57 head were fed for 114 days in the winter of 1928-29. They were of Hereford and mixed Hereford and Brahman breeding, bred and raised under the same conditions as those described in Texas Bulletins Nos. 385 and 409. In these lots were included all steers born at the Ranch Experiment Station in 1927 and 1928. The 1927 steers were approximately 19 to 22 months old when the formal feeding period began, while the 1928 steers were about 7 to 10 months old. The average daily gain per head for all lots was 2.53 pounds, with an average intralot standard deviation (51 degrees of freedom) of 0.398 pound, thus giving a coefficient of variability of 15.8 per cent, which is larger than was found in the other experiments, although still below the average of 17 per cent reported by Mitchell and Grindley (8).

Only two men took part in this experiment. Estimates of value per pound of live weight when finished were also made. The results are shown in Table 7. *A* and *B* denote the estimates of gain; *a* and *b*, the estimates of value per pound of live weight when finished; *I*, initial weight; *G*, gain; *P*, appraised value of dressed meat; and *V*, meat value of finished steer per pound of live weight.

TABLE 7.—Correlation coefficients from third feeding experiment with steers

Symbol <sup>a</sup>	Correlation coefficients for —						Average by Fisher's method
	Steers born in 1927			Steers born in 1928			
	Herefords, 4 head	First-cross Brah-mans, 13 head	Back crosses, 9 head	Herefords, 6 head	First-cross Brah-mans, 11 head	Back crosses, 14 head	
<i>r</i> <sub>AG</sub> -----	+0.28	+0.53	+0.53	+0.36	+0.22	-0.01	+0.30
<i>r</i> <sub>BG</sub> -----	+0.64	+0.63	+0.59	+0.48	+0.20	+0.03	+0.36
<i>r</i> <sub>IG</sub> -----	+0.93	+0.57	+0.46	-0.00	+0.12	+0.32	+0.36
<i>r</i> <sub>AI</sub> -----	+0.19	+0.86	+0.61	+0.86	+0.92	+0.29	+0.72
<i>r</i> <sub>BI</sub> -----	+0.80	+0.55	+0.59	+0.79	+0.84	+0.46	+0.62
<i>r</i> <sub>AB</sub> -----	-0.42	+0.59	+0.34	+0.93	+0.73	-0.22	+0.42
<i>r</i> <sub>AP</sub> -----	-0.45	+0.48	-0.36	-0.67	+0.37	+0.66	+0.29
<i>r</i> <sub>BP</sub> -----	+0.89	+0.13	-0.51	-0.26	+0.38	+0.28	+0.12
<i>r</i> <sub>IP</sub> -----	+0.85	+0.76	-0.74	+0.10	+0.44	-0.06	+0.20
<i>r</i> <sub>AV</sub> -----	-0.65	+0.40	-0.44	+0.23	+0.71	+0.59	+0.37
<i>r</i> <sub>BV</sub> -----	+0.76	+0.15	-0.26	+0.39	+0.68	+0.18	+0.26
<i>r</i> <sub>IV</sub> -----	+0.48	+0.74	-0.44	+0.82	+0.76	-0.12	+0.40
<i>r</i> <sub>AI</sub> -----	-0.80	+0.33	+0.46	+0.62	+0.68	+0.12	+0.36
<i>r</i> <sub>BI</sub> -----	-0.04	+0.44	+0.82	+0.80	+0.79	+0.12	+0.55
<i>r</i> <sub>AB</sub> -----	-0.00	+0.13	+0.10	+0.65	+0.66	+0.14	+0.29

<sup>a</sup> See text for explanation.

The averages in the last column are each based on 39 degrees of freedom; that is, they are as reliable as simple correlations calculated upon a group of 42 steers. The results are similar to those from the previous experiments except that the agreement between the men



was less in this instance. The correlations with gain are distinctly less than those between judgment and initial weight. The correlations with gain are on the border line of statistical significance when considered singly, but must be regarded as significant since all three are of the same sign and about the same magnitude.

The multiple correlation between gain as the dependent variable and *A*, *B*, and *I* as independent variables is 0.407, and this falls only to 0.397 when initial weight is left out of consideration. A little more than 16 per cent of the things which caused the differences in gains were seen by one or both men or were associated with differences in initial weight. The first-order partial correlation coefficients indicate again, as in the previous experiments, that much of what was seen by one man was also seen by the other or was reflected in initial weight.

Both men had some success in estimating the final value of the steers, one being more and the other less successful with value than with gain. Both had more success in estimating *V* than they would have had if the only causal connection had been through *P*; that is, they apparently took future dressing percentage into consideration with some slight degree of success.

The multiple correlation between *V* as the dependent variable and the two judgments and initial weight as independent variables was 0.472. The things which one or both men saw or which were associated with initial weight determined about 22 per cent of the differences in final value per pound of live weight but less than 10 per cent of the differences in appraised value of the dressed meat. When initial weight is left entirely out of consideration the men together saw 16 per cent of the causes of differences in final value per pound of live weight and nearly 9 per cent of the causes of differences in the appraised prices of the dressed meat.

#### FOURTH EXPERIMENT WITH STEERS

Forty steers were fed for 150 days at College Station during the winter of 1928-29. They were used in a study of market grades and were purchased on the Fort Worth market, 10 head to represent each of the feeder grades—Choice, Good, Medium, and Common. Their previous history was only slightly known, but at the beginning of the experiment they appeared to be about 10 to 15 months old and their average weight was 523 pounds. The average daily gain per head for the entire group was 2.35 pounds. The corresponding average intralot standard deviation (36 degrees of freedom) was 0.287 pound, thus giving a coefficient of variation of 12.2 per cent. The gains of these steers were more uniform than the average found by Mitchell and Grindley (8).

Eleven men estimated independently the gains for each lot. No attempt was made to estimate final values. The results are shown in Table 8. *I* indicates initial weight and *G* indicates gain. The other letters indicate the estimates of the various men. All further calculations are based upon the average correlations in the last column of the table.

TABLE 8.—*Correlation coefficients from fourth feeding experiment with steers*

Symbols <sup>a</sup>	Correlation coefficients for—				Averages by Fisher's method
	Choice feeder steers	Good feeder steers	Medium feeder steers	Common feeder steers	
TGA.....	-0.07	+0.35	-0.04	+0.31	+0.10
TGB.....	-.35	+.30	-.41	-.02	-.12
TGC.....	-.22	+.40	-.02	-.13	+.01
TGD.....	-.54	+.54	-.55	+.14	-.11
TGE.....	-.44	+.16	-.44	-.07	-.20
TGF.....	-.26	+.61	-.17	+.40	+.16
TGH.....	-.49	+.88	-.49	+.34	+.16
TGJ.....	-.38	+.55	+.35	+.49	+.26
TGK.....	-.63	+.50	-.34	+.66	+.06
TGL.....	-.26	+.44	-.50	+.38	+.01
TGM.....	-.20	+.70	-.36	+.19	+.09
TGI.....	-.62	+.67	-.34	+.09	-.04
TIA.....	+.69	+.17	+.24	+.56	+.34
TIB.....	+.63	+.48	+.47	+.77	+.58
TIC.....	+.16	+.76	+.87	+.21	+.57
TID.....	+.80	+.89	+.64	+.64	+.75
TIE.....	+.69	+.69	+.52	+.47	+.58
TIF.....	+.56	+.85	+.61	+.74	+.69
TIH.....	+.86	+.77	+.59	+.28	+.66
TIJ.....	+.85	+.39	+.12	+.73	+.53
TIK.....	+.85	+.81	+.67	+.48	+.71
TIL.....	+.71	+.68	+.67	+.61	+.65
TIM.....	+.52	+.80	+.57	+.20	+.55

<sup>a</sup> See text for explanation.

The average correlation between initial weight and the 11 sets of estimates is +0.629, whereas the average correlation between gain and the 11 sets of estimates is +0.039. The average correlation between one judge's estimates and the estimates of the other judges is +0.491. The same steers were used again and again in the correlations entering into these three averages. A glance at Table 8 shows that all correlations involving gain were negative—many of them strongly so—for the choice steers, all were positive for the good steers, all but one were negative for the medium steers, etc. The tendency here shown for all judges to do well with one lot and poorly with another was present to a lesser degree in the earlier experiments. If there happened to be some exceptional steers in the Choice lot or in the Good lot, the mere multiplication of judges who evidently have much the same ideal in mind would not add much to the general applicability of the figures. The number of steers needs also to be increased. Doubtless the averages are more reliable than those in the last column of Table 8, but less reliable than if an equal number of judges had worked on a much larger number of steers, and each steer had been used for the estimates by only two men. The distribution of the 220 original uncorrected correlation coefficients between the estimates of each man and those of every other man for each lot of 10 steers was as follows:

-0.50 to -0.59.....	1	+0.20 to +0.29.....	13
-0.40 to -0.49.....	1	+0.30 to +0.39.....	28
-0.30 to -0.39.....	1	+0.40 to +0.49.....	22
-0.20 to -0.29.....	9	+0.50 to +0.59.....	31
-0.10 to -0.19.....	4	+0.60 to +0.69.....	33
-0.00 to -0.09.....	4	+0.70 to +0.79.....	30
+0.00 to +0.09.....	5	+0.80 to +0.89.....	19
+0.10 to +0.19.....	14	+0.90 to +0.99.....	5

The averages in the last column of Table 9 need to be larger than 0.35 if the probability of their being accidental is to fall below 0.05. On that basis, not one of the correlations with gain is significant, but practically all of the correlations between initial weight and the estimates are significant, and so also are nearly all the correlations between 1 man's estimates and the estimates of the other 10 men. This is quite in accord with the previous experiments except that here the correlations with gain average close to zero.

When the average correlations in the last column of Table 8 are used, with gain as the dependent variable and initial weight and the 11 judgments as the independent variables, the multiple correlation coefficient is +0.736, but this sums up everything associated with gain, positively or negatively, and whether seen by only 1 man or by all 11. It should almost inevitably be rather large even if no one man by himself was reasonably successful in his estimates. A circumstance which may have had an important bearing on the outcome of this experiment was that the steers used were selected on the public market for their conformity to the standard market grades of feeder steers. They may not have been raised on the same ranch or even in the same region. Because they were selected for their conformity to the standard market grades, the steers within each lot were doubtless more uniform in outward appearance than those in the other experiments but less uniform in previous treatment. That this greater uniformity in outward appearance did not result in much, if any, greater uniformity of gains may be seen by comparing the coefficient of variability of their gains (12.2 per cent) with those in the first three experiments (10.6, 12, and 15.8 per cent). All but three lots of the steers in the first three experiments were bred and raised at the Texas station on one 5-section ranch and were subjected to the same treatment in feeding practices and in handling.

#### FIFTH EXPERIMENT WITH STEERS

Feeding of 40 steers was begun at College Station late in September, 1929, to repeat the preceding year's experiment. One died of anthrax during the experiment. The steers were purchased on the Fort Worth market, 10 head to represent each of the feeder grades—Choice, Good, Medium, and Common. Their previous history was unknown, but they appeared to be about 10 to 15 months old and averaged 535 pounds at the beginning of the experiment. The average daily gain per head for the entire group of 39 for 157 days was 2.21 pounds. The corresponding average intralot standard deviation (35 degrees of freedom) was 0.330 pound, thus giving a coefficient of variation of 14.9 per cent. The gains made by these steers were slightly more uniform than the average found by Mitchell and Grindley (8).

Ten men on the college, station, and extension staffs estimated independently the gain for each lot, and they estimated also what the steers would be worth per pound on foot at the end of the feeding period. A group of 10 senior students in animal husbandry who were trying out for the judging team used these steers for judging practice just before the beginning of the feeding period and made estimates in the same manner as the staff members. The results of the faculty estimates are shown in Tables 9 and 11 and those of the

students in Tables 10 and 12. *G* indicates gain; *I* initial weight; and *V*, final value per pound of live weight. These final values are thought to be slightly less accurate than those in the second and third steer experiments because these were based upon a single appraisal rather than on the average of three independent appraisals of the dressed meat.

TABLE 9.—Correlation coefficients from fifth feeding experiment with steers

(Staff members' estimates of gain)

Symbol <sup>a</sup>	Correlation coefficient for—				
	Choice feeder steers	Good feeder steers	Medium feeder steers	Common feeder steers	Averages by Fisher's method
TGA-----	+0.40	+0.26	+0.45	+0.32	+0.34
TGB-----	+.71	+.04	-.04	-.32	+.29
TGC-----	+.34	+.14	+.08	+.24	+.19
TGD-----	+.46	+.04	+.38	+.39	+.30
TGE-----	+.55	-.45	+.46	-.16	+.09
TGF-----	+.59	+.26	+.17	+.43	+.35
TGH-----	+.54	+.08	+.18	-.20	+.14
TGI-----	-.19	-.27	+.61	+.82	+.34
TGK-----	+.29	+.14	+.37	+.10	+.21
TGL-----	+.23	-.28	+.55	-.13	+.10
TGI-----	+.41	+.15	+.17	-.17	+.13
TIA-----	+.88	+.36	+.34	+.68	+.59
TIB-----	+.84	+.46	+.75	+.42	+.63
TIC-----	+.79	+.68	+.57	+.67	+.66
TID-----	+.85	+.72	+.87	+.79	+.80
TIE-----	+.66	+.68	+.75	+.48	+.60
TIF-----	+.63	+.82	+.74	+.54	+.68
TIH-----	+.88	+.77	+.85	+.44	+.75
TIJ-----	+.52	+.64	+.27	-.11	+.34
TIK-----	+.85	+.76	+.87	+.87	+.83
TI L-----	+.84	+.64	+.85	+.96	+.85

<sup>a</sup> See text for explanation.

Each correlation shown in the last column of Tables 9 to 12 is based on 27 degrees of freedom. The average of the 10 average correlations between initial weight and estimates is +0.694, whereas the average of the correlations between gain and the estimates is +0.237. The average correlation between one man's estimate and the estimates of the other men is +0.583. The distribution of the 180 original uncorrected correlation coefficients between the estimates of each man and those of every other man for each lot of steers was as follows:

-0.30 to -0.39-----	2	+0.30 to +0.39-----	15
-0.20 to -0.29-----	2	+0.40 to +0.49-----	18
-0.10 to -0.19-----	1	+0.50 to +0.59-----	22
-0.00 to -0.09-----	2	+0.60 to +0.69-----	35
+0.00 to +0.09-----	2	+0.70 to +0.79-----	33
+0.10 to +0.19-----	8	+0.80 to +0.89-----	23
+0.20 to +0.29-----	11	+0.90 to +0.99-----	6

The averages in the last column of Table 10 need to be larger than +0.35 if the probability of their being accidental is to be regarded as less than 0.05. Only three of the average correlations between gain and estimate come close to that level of significance. However, all 10 of those correlations are positive and the average of 0.237 between the actual gains and all estimates seems to prove that the positive

correlation between estimate and actual gain was real even though small.

The much higher correlations found between estimates of gain and initial weight than between estimates of gain and actual gain are in harmony with the results of previous experiments. The partial correlations between estimate and gain are only slightly smaller than the corresponding primary correlation coefficients. The partial correlations between initial weight and gain, the various judgments being constant, are distinctly smaller than the corresponding primary coefficients. These findings indicate that differences in initial weight rested on many things, only a few of which were associated with gain. Those few which were associated with gain were also seen by the men. The men saw in addition a small number of other things not associated with initial weight but associated with gain, and they also laid emphasis on many things associated with initial weight which were not actually associated with gain. Hence the high correlation between estimate and initial weight.

The multiple correlation coefficient between gain as the dependent variable and the 10 estimates and initial weight as the independent variables was 0.628. That the net contribution of initial weight to the forecasting of gains is practically zero in this case is indicated by the fact that the multiple correlation coefficient when initial weight is omitted falls from 0.628 only to 0.624.

The primary uncorrected correlation coefficients between the estimates of the 10 students and gain and initial weight are shown in Table 10, together with averages for all four lots calculated by Fisher's method.

TABLE 10.—Correlation coefficients from fifth feeding experiment with steers  
(Student estimates of gain)

Symbol <sup>a</sup>	Correlation coefficients for—				Averages by Fisher's method
	Choice feeder steers	Good feeder steers	Medium feeder steers	Common feeder steers	
$r_{GM}$ .....	+0.29	-0.28	+0.56	-0.14	+0.11
$r_{GN}$ .....	+0.22	-0.25	+0.59	+0.15	+0.19
$r_{GO}$ .....	+0.13	-0.40	-0.25	-0.31	-0.22
$r_{GP}$ .....	+0.34	-0.51	+0.31	-0.45	-0.10
$r_{GQ}$ .....	-0.09	-0.32	+0.56	-0.41	-0.05
$r_{GE}$ .....	-0.00	-0.38	+0.14	-0.38	-0.19
$r_{GS}$ .....	+0.23	+0.21	-0.19	+0.46	+0.17
$r_{GT}$ .....	-0.07	-0.16	+0.23	-0.21	-0.06
$r_{GU}$ .....	+0.65	-0.03	+0.48	-0.49	+0.15
$r_{GW}$ .....	+0.21	-0.40	+0.48	0.00	+0.07
$r_{IN}$ .....	+0.60	+0.55	+0.38	+0.72	+0.55
$r_{IO}$ .....	+0.57	+0.41	+0.21	+0.41	+0.38
$r_{IP}$ .....	+0.59	+0.39	+0.29	+0.74	+0.50
$r_{IQ}$ .....	+0.65	+0.63	+0.60	+0.52	+0.58
$r_{IR}$ .....	+0.48	+0.64	+0.71	+0.54	+0.58
$r_{IS}$ .....	+0.49	+0.42	+0.11	+0.53	+0.37
$r_{IT}$ .....	+0.53	+0.48	+0.31	+0.56	+0.45
$r_{IU}$ .....	+0.47	+0.49	+0.56	+0.57	+0.62
$r_{IW}$ .....	+0.60	+0.78	-0.08	+0.35	+0.45
	+0.61	+0.75	+0.24	+0.61	+0.55

<sup>a</sup> See text for explanation.

The average correlation between the gains and the estimates by the students was +0.007, or practically zero. This is slightly and

perhaps significantly smaller than the similar correlation found for the staff estimates. The students seemed to attach considerable importance to the things associated with initial weight, but the average correlation between their estimates and initial weight was only  $+0.51$  as contrasted with  $+0.69$  for the staff estimates and initial weight. The average correlation between the estimates of one student and those of all other students was  $+0.597$  as compared with a corresponding figure of  $+0.583$  for the average correlation between staff estimates.

The partial correlation coefficients between gains and estimates, initial weight being constant, differ only slightly from the primary correlation coefficients between estimate and gain. However, the partial correlation coefficients between gain and initial weight, the various estimates being constant, are somewhat larger in the case of the student estimates, which indicates that some of the things reflected in initial weight were not completely taken into account by the students when they made their estimate.

The multiple correlation coefficient between gain, as the dependent variable and initial weight and the 10 sets of estimates by students as the independent variables was  $0.891$ , which is very distinctly higher than the corresponding coefficient for the staff estimates. The estimates by the students, which were individually less accurate than those of the staff members, were decidedly more accurate when combined in the multiple correlation coefficient. This seems to mean that the staff members agreed very well with one another in regard to points which actually did have something to do with gain but disagreed on things which were not associated with gain. The total agreement of the students with one another was practically the same as for the staff members, but agreement seemed to be largely on things not actually associated with gain. The students differed in regard to a number of things, some of which were associated positively and some negatively with gain. Thus the combined result of estimating the gain according to their various estimates (giving negative weight to some sets of estimates, of course) is to include all things seen by any of the men regardless of whether they were seen by the others. The accuracy of the staff estimates improved only a little by including things seen by other members of the group, whereas the accuracy of the student estimates increased greatly when to what each student saw was added what every other student saw, whether that other student had interpreted it rightly or wrongly. That initial weight by itself has little importance is shown by the fact that the multiple correlation between gain and the 10 student estimates is  $0.880$  as compared with  $0.891$  when initial weight was also included. Not many things actually associated with initial weight escaped the notice of all 10 of the students making these estimates. The 180 original uncorrected correlations between one student's estimate and the estimate of other students were distributed as follows:

$-0.50$ to $-0.59$ .....	4	$+0.20$ to $+0.29$ .....	7
$-0.40$ to $-0.49$ .....	0	$+0.30$ to $+0.39$ .....	12
$-0.30$ to $-0.39$ .....	1	$+0.40$ to $+0.49$ .....	18
$-0.20$ to $-0.29$ .....	1	$+0.50$ to $+0.59$ .....	24
$-0.10$ to $-0.19$ .....	3	$+0.60$ to $+0.69$ .....	26
$-0.00$ to $-0.09$ .....	6	$+0.70$ to $+0.79$ .....	22
$+0.00$ to $+0.09$ .....	5	$+0.80$ to $+0.89$ .....	32
$+0.10$ to $+0.19$ .....	6	$+0.90$ to $+0.99$ .....	13

This distribution, like the others, shows very well the asymmetric distribution of correlation coefficients calculated from small samples where the true value of the correlation coefficient is fairly large.

In this experiment, as in the second and third steer experiments, an attempt was made to predict what the steers would be worth per pound on foot when the feeding was ended. The correlations between the estimates by the staff members and final value and initial weight and gain are shown in Table 11.

TABLE 11.—Correlations between final values and estimates of final values made by staff members at the beginning of the feeding period, fifth experiment with steers

Symbol <sup>a</sup>	Correlation coefficients concerning final values for—				Averages by Fisher's method
	Choice feeder steers	Good feeder steers	Medium feeder steers	Common feeder steers	
<i>Tv</i> a.....	+0.16	-0.13	+0.61	+0.59	+0.33
<i>Tv</i> b.....	+0.31	+0.19	+0.04	+0.37	+0.22
<i>Tv</i> c.....	+0.44	+0.58	+0.61	+0.27	+0.47
<i>Tv</i> d.....	+0.51	+0.38	+0.29	+0.70	+0.47
<i>Tv</i> e.....	+0.39	-0.18	+0.51	-0.12	+0.13
<i>Tv</i> f.....	+0.26	+0.50	+0.76	+0.77	+0.40
<i>Tv</i> h.....	+0.54	+0.35	+0.06	+0.44	+0.48
<i>Tv</i> i.....	-0.03	+0.50	+0.83	+0.11	+0.43
<i>Tv</i> l.....	+0.55	+0.15	+0.57	+0.13	+0.34
<i>Tv</i> l.....	+0.45	-0.06	+0.09	+0.63	+0.11
<i>Tv</i> g.....	+0.71	+0.31	+0.43	+0.20	+0.40
<i>Tv</i> i.....	+0.38	-0.35	-0.21	+0.54	+0.09
<i>Tv</i> a.....	+0.47	+0.39	+0.48	+0.47	+0.43
<i>Tv</i> b.....	+0.06	+0.01	+0.15	+0.01	+0.05
<i>Tv</i> c.....	-0.32	-0.14	-0.67	-0.22	-0.34
<i>Tv</i> d.....	+0.74	+0.20	+0.62	+0.77	+0.59
<i>Tv</i> e.....	+0.36	+0.19	+0.62	+0.29	+0.36
<i>Tv</i> f.....	+0.54	+0.46	+0.01	+0.38	+0.34
<i>Tv</i> h.....	+0.71	+0.32	+0.41	+0.42	+0.45
<i>Tv</i> i.....	-0.19	-0.35	-0.09	-0.34	-0.23
<i>Tv</i> l.....	+0.63	+0.43	-0.03	+0.39	+0.35
<i>Tv</i> l.....	+0.84	+0.64	+0.85	+0.88	+0.80
<i>Tv</i> g.....	+0.01	-0.04	+0.38	+0.60	+0.25
<i>Tv</i> b.....	+0.23	-0.27	+0.18	-0.18	-0.02
<i>Tv</i> c.....	+0.28	+0.05	-0.06	-0.44	-0.06
<i>Tv</i> d.....	+0.54	-0.11	+0.67	-0.03	+0.28
<i>Tv</i> e.....	+0.29	-0.15	+0.36	-0.13	+0.09
<i>Tv</i> f.....	+0.34	-0.13	+0.18	+0.25	+0.15
<i>Tv</i> h.....	+0.71	+0.09	+0.32	-0.31	+0.21
<i>Tv</i> i.....	-0.52	-0.03	+0.09	+0.01	+0.05
<i>Tv</i> l.....	+0.48	+0.03	+0.03	-0.21	+0.07
<i>Tv</i> l.....	+0.23	-0.28	+0.55	-0.17	+0.09

<sup>a</sup> See text for explanation.

The correlations between final value and estimates show the usual variation characteristic of correlations calculated from small samples. However, the average correlation between actual and estimated final values was positive for each set of estimates and the average of these 10 averages was 0.344, which is 0.107 higher than the corresponding figure for the estimates of gain by the same men. This difference is of doubtful significance. However, the difference found here is the same as was found in the second and third steer experiments, and is in line with what would be expected from professional animal husbandmen who had had much more practice in selecting steers that would be desirable for butchering than in picking those that would make large gains. As in the other two experiments, the men showed much less agreement among themselves in estimating final values than in estimating

gains. The average of the correlations between estimates made by each man and each of the other men is +0.350. The distribution of the 180 uncorrected correlations between one estimate and another for each lot is as follows:

-0.40 to -0.49	2	+0.30 to +0.39	19
-0.30 to -0.39	5	+0.40 to +0.49	20
-0.20 to -0.29	5	+0.50 to +0.59	19
-0.10 to -0.19	9	+0.60 to +0.69	14
-0.00 to -0.09	10	+0.70 to +0.79	23
+0.00 to +0.09	15	+0.80 to +0.89	4
+0.10 to +0.19	17	+0.90 to +0.99	1
+0.20 to +0.29	17		

The average correlation between initial weight and the estimates was +0.317. The partial correlation coefficients between estimates and final value, initial weight being constant, were practically the same as the corresponding primary coefficients. Whatever success these men had in picking the steers with the high and low final value was not noticeably influenced by whether or not they tended to pick large or small steers in their estimating.

The multiple correlation coefficient between final value as the dependent variable and the 10 estimates of final value and initial weight and gain as the 12 independent variables is 0.811. This falls only to 0.792 when gain is left out and to 0.762 when initial weight is also left out.

The steers used in this experiment were selected because of their close conformity to the market grade which each lot was to represent. It is therefore to be expected that there would be more uniformity in the final value of the steers within each lot than would be the case in many other experiments. The importance of this fact in explaining the actual results may be partly measured by the size of the coefficient of variability for the final values within each lot. The average final value for all 39 steers was 11.11 cents per pound and the average intralot standard deviation (35 degrees of freedom) was 0.68 cent per pound, giving a coefficient of variability of 6.14 per cent. This figure is distinctly lower than that found for the variability of gain, but is actually higher than in the second and third steer experiments, where there was no such rigid selection for feeder grade at the beginning of the experiment. An additional clue is perhaps furnished by the average values between lots, which were: Choice steers, 12.43 cents per pound; good steers, 11.66; medium steers, 10.41; and common steers, 10.05. The variance per degree of freedom between lots is nearly three times as great as it is within lots, thus indicating (although the amount of data is too small to be considered convincing) that a very large portion of the differences in final value between the whole group of four lots of steers had been seen in advance and discounted when the steers were divided into lots according to their feeder grade.

The correlation studies reported here bear only on those differences in final value which existed between steers in the same lot. Therefore they fail to show how much of the variation in final market value of all kinds of steers could have been predicted by these men. These correlation studies do indicate, however, that between steers given the same market grade as feeders there were still a few small differ-



ences which could be foreseen by the men who studied them. Of course in most ration tests the steers used would all come within the same market grade or close to it.

The student estimates of final value had a slightly lower correlation with the actual values than the estimates made by the staff members, although the difference is so small that it does not even approach significance in the statistical sense of the word. The original and average correlations between the student estimates and final value and gain and initial weight are shown in Table 12.

TABLE 12.—Correlations between final values and estimates of final values made by students at the beginning of the feeding period, fifth experiment with steers

Symbol *	Correlation coefficients concerning final values for—				Averages by Fisher's method
	Choice feeder steers	Good feeder steers	Medium feeder steers	Common feeder steers	
<i>FFm</i> .....	+0.03	-0.45	+0.22	+0.64	+0.13
<i>FFn</i> .....	+0.14	-0.13	+0.78	+0.85	+0.52
<i>FFo</i> .....	+0.16	-0.08	+0.52	+0.75	+0.37
<i>FFp</i> .....	+0.16	-0.60	+0.17	+0.71	+0.13
<i>FFq</i> .....	+0.14	-0.32	+0.66	+0.57	+0.30
<i>FFr</i> .....	-0.20	-0.25	+0.74	+0.48	+0.25
<i>FFs</i> .....	+0.16	-0.45	+0.53	+0.65	+0.25
<i>FFt</i> .....	-0.09	-0.30	+0.69	+0.76	+0.33
<i>FFu</i> .....	+0.44	-0.55	+0.03	+0.63	+0.14
<i>FFv</i> .....	+0.10	-0.64	+0.77	+0.58	+0.24
<i>FFw</i> .....	+0.45	+0.26	+0.15	+0.70	+0.40
<i>FIa</i> .....	+0.36	+0.23	-0.27	+0.47	+0.19
<i>FIb</i> .....	+0.56	+0.29	+0.11	+0.71	+0.42
<i>FIc</i> .....	+0.52	+0.66	+0.71	+0.30	+0.55
<i>FIq</i> .....	+0.57	+0.55	+0.03	+0.25	+0.35
<i>FIr</i> .....	+0.45	+0.29	-0.30	+0.24	+0.15
<i>FIe</i> .....	+0.43	+0.38	+0.13	+0.56	+0.37
<i>FIi</i> .....	+0.38	+0.34	+0.20	+0.78	+0.49
<i>FIu</i> .....	+0.56	+0.67	+0.43	+0.75	+0.57
<i>FIv</i> .....	+0.57	+0.57	-0.16	+0.56	+0.39
<i>FGm</i> .....	+0.07	-0.29	+0.57	-0.35	+0.01
<i>FGa</i> .....	+0.43	-0.50	+0.31	+0.12	+0.05
<i>FGc</i> .....	+0.10	-0.40	-0.20	-0.31	-0.21
<i>FGp</i> .....	-0.05	-0.61	+0.21	-0.20	-0.18
<i>FGq</i> .....	+0.19	-0.20	+0.70	-0.42	+0.09
<i>FGr</i> .....	-0.19	-0.49	-0.06	-0.01	-0.19
<i>FGs</i> .....	+0.29	-0.22	-0.00	-0.05	-0.01
<i>FGt</i> .....	-0.10	-0.38	+0.21	-0.30	-0.14
<i>FGu</i> .....	+0.61	-0.09	+0.39	-0.16	+0.18
<i>FGv</i> .....	+0.16	-0.52	+0.11	-0.16	-0.12

\* See text for explanation.

The average correlation between student estimates and actual final values was +0.270, while that between estimates and initial weight was +0.395. The distribution of the 180 original uncorrected correlation coefficients between one set of estimates and another set is as follows:

-0.40 to -0.49	1	+0.30 to +0.39	18
-0.30 to -0.39	0	+0.40 to +0.49	17
-0.20 to -0.29	1	+0.50 to +0.59	25
-0.10 to -0.19	0	+0.60 to +0.69	26
-0.00 to -0.09	3	+0.70 to +0.79	23
+0.00 to +0.09	9	+0.80 to +0.89	33
+0.10 to +0.19	10	+0.90 to +0.99	7
+0.20 to +0.29	7		

The tendency of the students to agree with each other in their estimates of the final value of steers was more pronounced than the corresponding tendency among the staff members, the two averages being  $+0.593$  and  $+0.350$ , respectively. This difference probably approaches significance, although in the absence of an entirely accurate means of estimating the degrees of freedom lost in using each estimate nine times in the comparisons with the other estimates, one is perhaps not justified in concluding that the difference is significant.

The multiple correlation coefficient between final value as the dependent variable and the 10 student estimates and initial weight and gain as the independent variables is  $0.774$ . When gain is omitted the multiple correlation coefficient with the other 11 independent variables falls to  $0.718$ . When initial weight is also left out of consideration and only the 10 sets of estimates are used as independent variables, the multiple correlation coefficient falls from  $0.718$  only to  $0.716$ . The first-order partial correlation coefficients show that practically all of the things associated with initial weight which were also associated with final value, were seen and taken into account by nearly all of the 10 students who made the estimates. The average correlation between the student estimates of final value and the actual gain was  $0.052$ .

#### DISCUSSION

The results of the eight experiments recorded in this paper agree so well that certain conclusions appear to be unavoidable in spite of the handicaps of extremely small lots and statistical methods not quite perfectly adapted to the problem. One of these conclusions is that a high correlation existed between initial weight and the estimates of gain. This averaged  $+0.904$  in the pig experiments and  $+0.699$  in the steer experiments. Presumably it is not weight as such which influences the judges, but the broader concept of size as indicated by bigness of frame and vigorous "growthy" appearance. It is not unusual to hear disparaging remarks about the average farmer's stock-judging ability because he frankly lays so much emphasis on size. And yet when it comes to estimating gains, these figures seem to show that the professional animal husbandman himself attaches considerable importance to size. That this is partially justified is shown by the rather general tendency of initial weight to be correlated with gain. However, this correlation was not found in the first pig experiment nor in the fourth steer experiment.

The correlation between initial weight and gain was much larger in the second and third pig experiments than in any of the steer experiments. In an unpublished study by H. H. Mitchell, of the Illinois station (to whom the writer is indebted for permission to mention the results), the same difference between pigs and steers is noted. Mitchell found a generally high and significant correlation between initial weight and gain in published reports of feeding experiments with pigs at many stations, and a generally low or quite insignificant correlation between initial weight and gain in those experiments with steers in which sufficient data were given to permit the calculation of this correlation. The reasons for the higher correlation in pig experiments are not clear, but the following points may have some bearing on the problem: (1) Pigs are usually born and raised at the station where they are fed, while most steers are

purchased, and sometimes those in a single experiment come from several different places; (2) pigs are distributed by litters and thereby achieve a more perfect balancing with respect to hereditary differences than is usually possible with steers; (3) the variability in age and size of pigs at the beginning of the experiments is often (though not always) greater than that in the groups of steers started on feed; (4) pigs have a relatively simple digestive system as compared to that of ruminants and their capacity for consuming feed may be more closely and directly related to their size than is the case with steers.

Severson and Gerlaugh (9) reported a correlation of only  $+0.036$  between initial weight and gain of 338 steers fed at the Pennsylvania station during eight years. Their data were all grouped together in a single table, and consequently were affected by heterogeneity of mean weights and mean gains for the various lots. Had a correction been made for this heterogeneity the correlation would almost certainly have been somewhat larger.

Data on seven lots of steers fed at the Texas station before the experiments herein reported were begun give an average correlation (by Fisher's method (2)) of  $+0.333$  (44 degrees of freedom) between initial weight and gain. All but 5 of the 67 steers used in the experiments were bred and raised at the station. When the data for these 67 steers are combined with the data presented in this study an average correlation of  $+0.240$  is found between initial weight and gain. This figure is based on data from 320 steers, but since they were fed in 33 separate lots and three degrees of freedom are lost for each additional lot into which they were separated, it is as reliable as if calculated on a single lot of 224 steers about as uniform as the average uniformity within each lot actually fed.

The correlation between initial weight and estimates of value was  $+0.420$  (three experiments only, but 798 degrees of freedom if each estimate is counted independently of other estimates made on the same steers), which is distinctly less than was found between initial weight and estimates of gain but is large enough to be of undoubted statistical significance. The average correlation between initial weight and actual final value was  $+0.386$  (111 degrees of freedom). The average correlation between estimates of final values and actual final values was  $+0.334$  (798 degrees of freedom, if each estimate is counted independently of other estimates made on the same steer). The average correlation between estimates of final value made by different men was  $+0.488$  (apparently 2,739 degrees of freedom but many of these were spurious because each estimate was used as many times as there were other estimates on the same steer), which is distinctly less than the corresponding correlations between estimates of gain. This rather slight agreement on the ideal type for high final value and fairly close agreement on the ideal type for large gains is all the more surprising because so much of the formal instruction in stock judging concerns the ideal butcher type and so little of it deals directly with the ideal feeder type. Nevertheless, although the men agreed with one another less closely in estimating final value than in estimating gain, they were slightly more successful in estimating final value.

It is conceivable that the usual variation in gains or in final value might be the sum of a rather constant unpredictable amount of variation and a certain variable amount which good judges would be

able to foresee. If this were true then the correlation between judgment and gain or final value should be high in those experiments in which gains or final values were quite variable and low in those experiments in which the gains or final values were very uniform. In other words, judges could foresee not a certain proportion of all variability in performance but a certain proportion (or all) of that above a minimum amount, which must be regarded as unpredictable. The data do not support this hypothesis, although, since only eight experiments were included, the evidence can hardly be considered conclusive. Thus, as Table 13 shows, the most successful estimates of pig gains were attained in the experiments in which the gains were most uniform, and the least successful estimates of steer gains were attained in the experiment in which gains were most uniform and in the experiment in which gains were least uniform. The degree of success in estimating final value does support this hypothesis fairly well, but the differences are small and only three experiments were included.

TABLE 13.—Summary of results from the eight experiments showing degree of success attained in forecasting individual gains and final values

EXPERIMENTS IN ESTIMATING GAINS

Description of experiment	Men making estimates	Data equivalent to calculations upon a single lot of animals	Percentage determination (100 $R^2$ ) of gain or final value—		Reduction in coefficient of variation if lots had been composed of animals identical in weight and in the eyes of all the men who made the estimates (from C. V. to C. V. $\sqrt{1-R^2}$ )
			By initial weight and all estimates	By initial weight and the one set of estimates having the highest primary correlation with gain or with final value	
	Number				From To
First with pigs .....	a 3	28	10.6	10.6	13.3-12.6
Second with pigs .....	3	48	50.0	46.6	9.8- 6.9
Third with pigs .....	(b) 3	61	49.3	46.9	12.1- 8.6
First with steers .....	1	43	8.8	8.8	10.6-10.1
Second with steers .....	4	48	18.3	15.2	12.0-10.8
Third with steers .....	2	42	16.6	16.2	15.8-14.4
Fourth with steers .....	11	31	54.2	11.3	12.2- 8.3
Fifth with steers .....	c 10	30	39.4	14.4	14.9-11.6
Fifth with steers .....	d 10	30	79.4	3.9	14.9- 6.8

EXPERIMENTS IN ESTIMATING FINAL MARKET DESIRABILITY

Second with steers .....	4	48	34.2	31.9	5.49-4.55
Third with steers .....	2	42	22.3	22.3	4.10-3.61
Fifth with steers .....	e 10	30	62.7	23.3	6.14-3.75
Fifth with steers .....	d 10	30	51.6	27.1	6.14-4.26

a As a committee.

b 4 with 3 repeating.

c Staff members.

d Senior students.

It will be seen from Table 13 that as a whole the success of the men in estimating gains was quite limited. In 4 experiments about one-half of all the causes of differences in gains were foreseen, in 2 experiments about one-sixth, and in 2 experiments about one-tenth. But in the second and third experiments with pigs, initial weight was so

closely associated with gain and with the judgments that the men would have had only a little additional success if they had been making estimates on a group of pigs already balanced according to weight.

The statistical procedure of combining in a multiple correlation coefficient the estimates of many different men where the lots were few and small and where average coefficients had to be used may not be perfectly valid. The method of averaging does not rest upon an absolutely rigid mathematical relation, and the approximate corrections for small size of the samples are based upon an average bias which will not prevail in every sample without exception. Probably no serious error is thereby introduced into the average simple correlation coefficients, but such errors might possibly be cumulative and become fairly large in such experiments as the third with pigs or the fourth and fifth with steers. To explore this possibility the figures in column 5 of Table 14 were prepared, using for independent variables initial weight and in each experiment that set of estimates which was most closely correlated with gain or with final value. This procedure, of course, will show more effect of balancing in most experiments than is justified, since the set of estimates to be used was picked out after the experiment was over.

The figures may be considered as representing the combined effect of balancing according to initial weight and according to the opinion of one unusually good judge. The differences between these figures and those in the preceding column are slight except for the gains in the fourth and fifth steer experiments and the final values in the fifth steer experiment (these were the only experiments in which more than four men made estimates). This tends to confirm the idea that the very high  $R^2$  found in those two experiments is misleading. Everything seen by even one man is included if correlated with the dependent variable, whether the correlation was in the direction the man thought or opposite to it. Naturally the inclusion of estimates by more and more men would almost inevitable result in additions to  $R^2$ .  $R^2$  might thus become very high without any one of the men having been really very successful in his estimates. This seems to have been exactly what happened with the student estimates of gain in the fifth steer experiment, in which many of the average primary correlation coefficients were negative. For these reasons it is believed that the figures in column 5 of Table 13 present a truer picture than do those in column 4. A larger figure for student estimates of gain would have been obtained in column 5 if the estimates of the student having the largest negative correlation with gain had been used instead of the estimates of the student having the largest positive correlation.

Only a small reduction in the amount of experimental error in the feeding trial would result if all the animals should weigh identically the same and be so nearly alike that the men in charge could not tell them apart. Apparently it is a rare experiment in which the variation under such extreme conditions would be reduced to much less than 75 or 80 per cent of what it actually is. More often the effects of balancing are so slight that the variation not foreseen or discounted in the balancing is at least 90 per cent as large as the gross observed variation. This of course does not mean that balancing should be omitted. On the contrary the fullest possible use should be made of

it. Anything it actually does accomplish is just so much gained and there is no possibility of doing harm by using it, even negative correlations between estimates and performance having a beneficial effect in helping to distribute the desired qualities equally. But it is important that we should not conclude that two lots are exactly comparable merely because careful and painstaking efforts were made to divide them so. It appears that in general the men saw the causes of gain which were associated with size and that they saw also a few of the other causes, but that most of the other causes of differences in gain were either not associated with any visible characteristics of the steers or the men did not know what those visible characteristics were.

Very little difference was found in the ability of various men to estimate gains or final values correctly. None of the differences found were statistically significant. However, the data were too few to prove the reality of small differences of this kind. Many of the differences observed in predicting ability seemed rather extreme, but were reversed on the next group of animals or in the next experiment. This conclusion, like the others, applies only to the range of data and of men included in this study. The estimating of gains and final values as practiced in these experiments was of course quite different from judging breeding animals as practiced at shows and fairs.

The question as to whether the men who made these estimates were representative of animal husbandmen in general can not of course be answered objectively. It may, however, be pointed out that the agricultural colleges at which they received their undergraduate training included seven—Kentucky, Indiana, Kansas, Ohio, Ontario, Wyoming, and Texas—and that one or more of them had done graduate work at the following institutions: Illinois, Iowa, Kansas, Missouri, Texas, and Wisconsin.

The coefficients of variation found in these eight experiments were in every case smaller than the average found by Mitchell and Grindley (8), in spite of the fact that the  $n-1$  formula used in calculating the standard deviations in this study tends to give slightly higher figures than would be obtained by the formula which they used. As a partial explanation of this fact it may be noted that all these gains were reasonably good ones, and of course a large gain is to some extent associated with a small coefficient of variability, inasmuch as the latter is a fraction of which the former is the numerator.

Finally, it should be repeated that one probable cause of variation in gains was outside the scope of this study; namely, variations in the amount of feed eaten. It is possible that this factor may have been the cause of all the observed correlations between initial weight and gain or between judgment and gain. If such were the case it would of course have a very important bearing on the significance of observed differences between lots, especially where differences in feed consumption are taken into account. The only means of attack on this problem seems (to the writer) to be an extensive series of individual feeding experiments paralleled by groups fed in the ordinary manner on the same rations as the individually fed animals and during the same periods.

## SUMMARY AND CONCLUSIONS

The relative gains of individual pigs and steers used in regular feeding experiments were estimated at the beginning of a series of experiments by members of the staff of the Texas Agricultural and Mechanical College and the agricultural experiment station. In three of the experiments with steers the men estimated also what the relative market desirability of the individual steers in each lot would be when fattening was completed. Both kinds of estimates were made relative to the performance of other individuals in the same lot, so as to eliminate the effects of changing price levels and of differences in the rations fed. The correlations found between those estimates, initial weights, and actual performance were then studied.

Differences in initial weight were only slightly correlated with differences in gain in the case of steers (average  $r = +0.24$ , based on the statistical equivalent of one lot of 224 steers), but they were distinctly correlated with gain in the case of pigs (average  $r = +0.52$ , based on the statistical equivalent of one lot of 128 pigs). Published data support the conclusion that the correlation between initial weight and gain is normally higher with pigs than with steers.

The average correlations between estimates and gains in the three pig experiments were:  $+0.05$ ,  $+0.63$ , and  $+0.64$ . The average correlations between estimates and gains in the five steer experiments were:  $-0.03$ ,  $+0.33$ ,  $+0.33$ ,  $+0.04$ ,  $+0.24$ , and (for students)  $+0.01$ . The average correlations between estimated and actual final values were  $+0.42$ ,  $+0.32$ ,  $+0.34$ , and (for students)  $+0.27$ .

The correlations mentioned in the two preceding paragraphs are large enough to be significant and to demonstrate the desirability of balancing lots at the beginning of experiments as carefully as possible, but they are not high enough to account for a very large fraction of the observed variations in gains or in final value.

The proportion (squared multiple correlation) of the causes of gain which were associated either with differences in initial weight or with things seen by the man who made the most successful set of estimates in a particular experiment ranged from 3.9 to 16.2 per cent for gains in the steer experiments, from 10.6 to 46.9 per cent for gains in the pig experiments, and from 22.3 to 31.9 per cent for final values in the steer experiments. Where the estimates of several men were included as additional independent variables the squared multiple correlation coefficients were larger of course, but much of this was due to the limited amount of data used.

The estimates were closely correlated with initial weight, especially in the experiments with pigs. Most of the men saw something more in the animals than was reflected in initial weight, but there were several cases of negative partial correlation coefficients between estimate and gain, initial weight being constant.

In three experiments where final value was estimated, the estimates were slightly more successful than the estimates of gain.

The most impressive finding in these experiments is the large amount of variation in gain and also in final value that was not foreseen by trained men who spent much time in close study of the experimental animals. Perhaps the major factors that determine which individuals will make large and which small gains, or which will be worth most and which will be worth least when the experiment is

over, are not after all closely associated with visible differences in the animals.

In the experimental work each lot was treated as a unit, complete within itself, and therefore the findings here reported apply only to the range of differences included within a single lot of animals. It is quite likely that higher correlations would have been found if each lot had included a wide range of animals varying greatly in size, age, and market grade, but such variations are not often found among animals in modern feeding experiments.

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# THE CALCIUM REQUIREMENTS OF DAIRY HEIFERS <sup>1</sup>

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## INTRODUCTION

For several years (1922-1928) investigations were carried on at this station to determine the value of mineral supplements in the rations of dairy cows. When a study was made of the literature in connection with this problem it was discovered that very little information is on record relative to the mineral requirements of dairy animals, more particularly of young stock. Because of this fact it was difficult to measure the adequacy in this respect of different types of rations. Accordingly a project was organized in 1927 the aim of which was to determine, if possible, the calcium and phosphorus requirements of dairy heifers from the time they are weaned from milk (5 to 6 months) until they freshen the first time (28 months or thereabouts). It was thought that at the very least it would be possible, with the results secured, to determine whether or not average rations which are fed in practice to dairy heifers carry sufficient mineral matter for proper growth.

## EXPERIMENTAL ANIMALS

The calcium requirements were the first to be investigated and for this purpose eight high-grade Holstein heifers were used.<sup>2</sup> They were divided into two groups; one group was fed a ration high in calcium, the other was fed a ration low in calcium. The history of these heifers is shown in Table 1.

TABLE 1.—*History of the heifers used in the calcium requirement tests*

Group	Heifer No.	Born	Age at commencement of experiment	First calf delivered	Age at first calving
			Days		Days
High-calcium ration.....	121	Aug. 30, 1926	136	Dec. 14, 1928	837
	<sup>a</sup> 125	Sept. 17, 1926	131		
	131	Jan. 27, 1927	278	June 20, 1929	875
	137	July 24, 1927	161	Oct. 30, 1929	829
	139	Aug. 5, 1927	149	Dec. 29, 1929	877
Average.....			<sup>b</sup> 144		855
Low-calcium ration.....	126	Oct. 2, 1926	132	Feb. 16, 1929	868
	<sup>c</sup> 128	Nov. 2, 1926	119	July 30, 1929	1,001
	138	July 29, 1927	156	Dec. 11, 1929	866
	141	Aug. 27, 1927	139	Dec. 22, 1929	848
Average.....			137		<sup>d</sup> 861

<sup>a</sup> Died at 13 months; No. 131 substituted for her.

<sup>b</sup> Average does not include No. 131.

<sup>c</sup> It was unfortunate that No. 128 calved at a much later age than any of the others. She was bred in the spring of 1928 and not showing heat three weeks later was presumed to be with calf, and was sent to pasture. It was not discovered that she was not with calf until she returned from pasture in September and some difficulty was then experienced in getting her bred.

<sup>d</sup> Average does not include No. 128.

<sup>1</sup> Received for publication Dec. 1, 1930; issued June, 1931. Contribution No. 112 of the Massachusetts Agricultural Experiment Station.

<sup>2</sup> One of these died of acute tympany at 13 months of age, and another heifer that had been held in reserve was substituted for her.

## EXPERIMENTAL FEEDING

The general nature of the rations was as follows:

High calcium ration	Low calcium ration
Alfalfa hay.	Hay from mixed grasses.
Some mixed hay.	Alfalfa (small amount, first year).
Dried beet pulp.	Dried apple pomace.
Low protein concentrate mixture (corn meal for the most part).	High protein concentrate mixture (usually corn meal supplemented with linseed meal).

During the first period of six months the high-calcium group received approximately two-thirds alfalfa and one-third mixed hay; during the second period, from September, 1927, to June, 1928, the roughage was largely alfalfa; during the third and fourth periods (October, 1928, to June, 1929, and September to December, 1929) alfalfa averaged about 60 per cent and mixed hay about 40 per cent of the hay ration.

The roughage for the low-calcium group consisted entirely of mixed hay except in the first period (March to June, 1927), when alfalfa constituted about one-third of the roughage. These adjustments were necessary in order to keep the amount of calcium fed to the two groups at the proper level.

## CHARACTER AND COMPOSITION OF FEEDS

The alfalfa was a California product of excellent quality. The hay was grown on the college grounds and consisted of a mixture of grasses. Beet pulp and the several concentrates composing the two mixtures were purchased in the open market and were of good average quality. The apple pomace was secured from a cider manufacturer in a near-by town and was dried with the aid of modern machinery. The composition of the various feeds is shown in Table 2.

TABLE 2.—Percentage composition of feeding material used in the experiments

Number of analyses	Material	Moisture			Nitrogen			Ash		
		Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
8	Low protein concentrate mixture.....	10.34	14.40	12.33	1.840	2.007	1.904	2.78	2.90	*2.86
5	High protein concentrate mixture.....	8.46	11.87	10.38	3.191	3.401	3.322	3.10	3.70	*3.42
15	Alfalfa hay.....	8.50	11.68	9.82	2.380	3.555	2.701	7.36	9.85	*8.27
17	Mixed hay.....	7.28	10.93	9.32	.890	1.789	1.205	4.60	6.28	*5.10
5	Dried apple pomace.....	5.85	11.85	9.50	.752	1.003	.846			*2.43
9	Dried beet pulp.....	7.88	12.25	9.93	1.225	1.577	1.432	3.65	3.95	*3.81

Number of analyses	Material	Calcium			Phosphorus			Magnesium		
		Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
8	Low protein concentrate mixture.....	0.028	0.070	0.048	0.379	0.432	0.414	0.124	0.190	0.157
5	High protein concentrate mixture.....	.055	.087	.073	.475	.563	.509	.202	.242	.210
15	Alfalfa hay.....	1.074	1.536	1.272	.218	.313	.247	.280	.480	.316
17	Mixed hay.....	.403	.537	.488	.110	.285	.176	.119	.209	.158
5	Dried apple pomace.....	.112	.197	.155	.080	.111	.099	.048	.060	.057
9	Dried beet pulp.....	.468	1.125	.843	.061	.101	.077	.232	.317	.281

\*6 determinations.    \*5 determinations.    \*7 determinations.    \*1 determination.    \*3 determinations.

The high-calcium ration supplied about twice as much of that element as did the low calcium-ration. An attempt was always made to keep the other constituents of both rations as nearly on a par as possible, although it was found somewhat difficult to do this in practice. The beet pulp was included partly because of its calcium content and partly to furnish succulence and palatability. The apple pomace served the latter purpose admirably in the low-calcium ration and carried very little mineral matter. The high protein content of the alfalfa was offset by feeding to the heifers in the low-calcium group a grain mixture somewhat higher in protein. For the most part this was done by simply adding linseed meal to their ration in small amounts. The standard used in adjusting the rations for protein and energy was that given by Armsby<sup>3</sup> for dairy cattle. For lack of something more specific Kellner's mineral standard<sup>4</sup> was used as a general guide in determining the levels of calcium to feed, but it can not be regarded as particularly applicable since it was devised for mature cows. It calls for a daily minimum intake of 3.25 gm. of calcium and 1 gm. of phosphorus for each 100 pounds of live weight. The detailed intake of nutrients by both groups at different periods throughout the experiment is set forth in Table 3.

TABLE 3.—*Intake of total nutrients by heifers, per 100 pounds live weight*

Period	Group	Intake (in pounds daily per 100 pounds live weight) of—						
		Dry matter	Protein	Crude fiber	Nitrogen-free extract	Fat	Calcium	Phosphorus
March-June, 1927.....	High calcium....	2.04	0.27	0.50	1.09	0.047	0.019	0.005
	Low calcium....	2.16	.29	.49	1.20	.069	.011	.007
September, 1927-June, 1928.....	High calcium....	1.69	.25	.33	.95	.043	.015	.004
	Low calcium....	1.73	.22	.42	.94	.059	.007	.005
September, 1928-June, 1929.....	High calcium....	1.46	.19	.31	.84	.035	.011	.003
	Low calcium....	1.60	.19	.37	.91	.057	.006	.004
September-December, 1929.....	High calcium....	1.51	.20	.34	.85	.042	.012	.004
	Low calcium....	1.57	.16	.37	.94	.057	.007	.004

The periods in Table 3 represent the intervals of time during which mineral balance trials were being carried on with the heifers. During July and August of each year the heifers were on pasture. The intake of digestible protein and the net energy during the above-mentioned periods are shown in Table 4.

TABLE 4.—*Intake of digestible protein by heifers and net energy per 100 pounds live weight*

Period	Group	Digestible protein (pounds daily)	Net energy (therms daily)
March-June, 1927.....	High calcium....	0.18	1.14
	Low calcium....	.20	1.23
September, 1927-June, 1928.....	High calcium....	.15	1.09
	Low calcium....	.15	1.06
September, 1928-June, 1929.....	High calcium....	.13	.91
	Low calcium....	.11	.95
September-December, 1929.....	High calcium....	.13	.90
	Low calcium....	.09	.92

<sup>3</sup> ARMSBY, H. P. THE NUTRITION OF FARM ANIMALS. Table IV (b), p. 713. New York, 1917.<sup>4</sup> KELLNER, O. DIE ERNÄHRUNG DER LANDWIRTSCHAFTLICHEN NUTZTIERE. Aufl. 8, Berlin, p. 618, 1919.

It will be noted that during the last two periods shown the low-calcium group received less digestible protein than the high-calcium group, the difference increasing somewhat as the animals became older. The difference was unavoidable since it was desired to supply both groups the same amount of net energy per unit of weight and at the same time keep the level of calcium intake, derived largely from alfalfa, somewhat higher than in the low-calcium group. To be explicit, as heifers approach maturity their protein requirements increase little, if any, while their energy requirements continue to increase. This creates a situation which renders accurate balancing of rations impossible where alfalfa hay, with its high protein content, is fed. In order to insure a sufficient intake of energy an excess of protein must be fed. It was necessary to feed alfalfa hay to the high-calcium group in order to keep the level of calcium intake somewhat higher than that in the low-calcium group; hence the spread between the two groups in intake of digestible protein, which became more noticeable as the average age of the heifers increased.

#### METABOLISM BALANCE TRIALS

Mention has already been made of the metabolism balance trials which were carried on. This phase of the investigation was perhaps the most important in the whole program. Eighty-four of these trials were carried through. During the trials the heifers were kept in specially constructed stalls arranged for convenient collection of urine and feces. These stalls were made adjustable so as to accommodate the heifers from the time they were small calves until they were full grown. This was accomplished by sliding the stanchion and manger backwards or forwards and by employing portable side pieces which were used when the animals were small and removed as they grew older, leaving the full width of the stall for the larger animals. (Fig. 1.) The animals were kept in the stalls from 12 to 14 days for each trial, the first 2 to 4 days being a preliminary period to allow them to become accustomed to the routine. The last 10 days of each period constituted the experiment proper, and during this time the urine and feces were collected daily. Each animal stood on heavy waterproofed sailcloth, under which, as an extra protection against loss of urine, there was placed a strip of rubber matting extending the full length and width of the stall. Underneath that was a layer of shavings about 2 to 3 inches thick. The floor of the stall was covered with galvanized sheet iron and the entire stall was given a slight pitch to the rear so as to insure free drainage into the collection hopper, which was also lined with galvanized iron. The hopper fitted snugly to the rear of the stall and was easily removable when the animals were put up or taken down. The urine and feces were collected in large galvanized-iron pails placed underneath the hoppers. Each morning at 8.30 during the course of a trial the pails were removed and weighed, and the urine and feces thoroughly mixed with a wire beater. A 10 per cent sample of the 24-hour output of each animal was then carefully taken, placed in a friction-cover tin pail, and taken at once to the laboratory where it was subsampled down to 1 per cent of the 24-hour output. The laboratory samples were preserved in glass-stoppered 2-liter jars with chloroform and refrigeration, each day's sample being added to the composite. At

the end of the period total nitrogen was determined at once on quadruplicate charges of the fresh composite sample, and triplicate charges were ashed in the wet way with sulphuric and nitric acids. The solutions thus obtained were preserved in volumetric flasks and later analyzed for calcium, phosphorus, and magnesium. All feeds were carefully sampled and analyzed, and all waste feed was accounted for, although it should be noted that very little feed was ever wasted.

The nitrogen balances were determined in order to have a criterion of normal growth. The magnesium balances were followed in order to ascertain if possible what interrelation, interdependence, or an-



FIGURE 1.—Rear view of metabolism stalls; the unoccupied stall has the removable side pieces in position

tagonism existed between the metabolism of this element and that of calcium.

Of the 84 trials completed, results from 76 were employed in making the final summary. Either because of known laboratory errors, or because of such abnormalities of the animals as poor appetite or foul smelling, very apparently abnormal feces, results from the other 8 have not been included. Table 5 shows the distribution of the trials between groups and individuals.

TABLE 5.—*Distribution of metabolism trials between groups and individual heifers*

Group	Number of times use was made of heifer No. —								
	121	125	131	137	139	126	128	138	141
High calcium.....	9	4	9	10	7	—	—	—	—
Low calcium.....	—	—	—	—	—	10	11	8	8

The detailed balance records for the individual heifers are given in Table 6. The summarized data appear in Table 7. Considerable fluctuations occurred in the amounts of the several elements stored. Oftentimes these were much greater in different trials with the same animal than between different animals in the same group or between the two groups.

TABLE 6.—Detailed balance record for each heifer in the mineral metabolism experiment

HIGH-CALCIUM GROUP

Heifer No.	Actual retention in grams daily of—				Retention per 100 pounds live weight in grams daily of—				Retention as percentage of intake of—			
	N	Ca	P	Mg	N	Ca	P	Mg	N	Ca	P	Mg
121.....	17.278	8.764	5.545	-0.133	4.320	2.191	1.386	-0.033	19.96	23.45	50.76	-1.17
	11.779	5.031	4.958	2.052	2.752	1.316	1.158	.479	13.51	14.96	45.17	18.19
	29.816	6.983	1.716	1.839	4.909	1.164	.286	.307	28.83	18.29	15.33	13.33
	21.882	1.496	2.697	-.084	3.678	.251	.453	-.014	21.16	3.92	24.09	-.60
	34.816	10.424	4.392	2.602	5.398	1.616	.681	.408	32.20	26.01	37.59	17.82
	17.417	7.794	3.639	-.085	2.219	.993	.464	-.011	16.11	19.45	31.14	-.58
	21.801	9.453	6.018	4.940	2.565	1.112	.708	.581	17.92	20.09	44.82	29.76
	16.143	14.523	2.279	-1.779	1.682	1.513	.237	-.183	12.58	30.83	15.47	-9.38
	19.179	7.260	1.813	-3.186	1.579	.598	.149	-.262	12.14	12.37	11.10	-13.12
Average.....	21.123	8.036	3.673	.685	2.935	1.117	.510	.095	18.92	18.82	29.48	4.60
125.....	10.692	7.118	4.851	-.269	3.240	2.157	1.470	-.082	14.19	22.77	49.52	-11.79
	10.496	5.760	5.081	1.420	2.957	1.623	1.431	.400	13.91	18.38	51.79	14.93
	16.386	5.598	4.885	-.020	3.707	1.287	1.123	-.005	19.07	15.10	45.37	-7.69
	27.855	3.689	1.855	.627	5.306	.703	.353	.119	27.74	10.16	16.82	4.77
Average.....	16.357	5.541	4.168	.440	3.977	1.347	1.103	.107	19.41	16.30	40.26	4.07
131.....	26.580	6.487	2.532	.359	5.842	1.426	.557	.079	29.17	18.30	24.83	2.97
	17.781	8.327	3.787	-.860	3.666	1.717	.777	-.177	19.51	23.49	36.94	-6.64
	15.678	11.572	3.599	3.738	2.703	1.995	.621	.644	14.74	26.43	34.62	25.58
	15.934	10.351	3.240	-1.923	2.276	2.336	.463	-.275	14.09	37.29	27.69	-11.23
	16.788	4.434	8.03	-1.521	1.908	.504	.091	-.173	13.30	11.15	6.07	-8.72
	31.349	2.157	4.756	-1.680	3.283	.226	.498	-.176	20.41	4.26	27.56	-8.06
	24.685	7.433	3.553	-3.641	2.329	.701	.335	-.843	15.26	14.18	19.15	-15.02
	34.491	4.188	3.712	-1.677	2.961	.359	.319	-.144	18.88	7.59	19.60	7.38
	35.077	6.128	1.586	-1.870	2.998	.524	.136	-.160	20.26	10.76	10.48	-.834
Average.....	24.263	7.453	3.061	-1.008	2.931	.900	.370	-.122	18.21	16.22	21.93	-4.16
137.....	24.179	4.610	4.895	5.076	7.218	1.376	1.461	1.515	26.67	13.30	53.98	42.63
	22.972	9.955	4.201	4.387	5.672	2.458	1.037	1.083	23.60	28.08	40.49	35.11
	14.911	14.189	3.301	-2.944	3.062	2.914	.678	-.605	15.32	10.86	31.82	-19.07
	32.490	3.370	3.323	-.433	4.303	.446	.440	-.057	26.92	8.84	27.37	-2.86
	25.898	5.387	2.891	-.528	2.977	.619	.392	-.061	20.08	12.66	20.29	-3.12
	17.232	6.285	3.882	-1.055	1.833	.669	.413	-.112	12.21	13.87	23.99	-5.65
	36.300	9.113	2.254	.738	3.821	.959	.237	.078	24.03	18.58	16.74	4.07
	50.626	5.606	4.635	-1.072	4.891	.542	.448	-.104	30.28	10.23	31.68	-5.13
	39.955	7.743	2.795	4.184	3.632	.704	.254	.380	24.15	12.50	14.93	17.51
	47.558	9.772	4.264	-.881	4.030	.828	.361	-.075	28.74	15.77	22.78	-3.56
Average.....	31.212	7.603	3.644	.747	3.874	.944	.452	.093	23.55	16.61	26.42	4.36
139.....	21.392	9.239	3.509	4.420	6.026	2.603	.988	1.245	24.50	26.80	40.51	38.06
	17.086	12.736	3.739	-1.466	4.206	3.145	.923	-.362	17.81	36.69	37.22	-10.61
	21.553	15.282	2.565	-.507	4.145	2.939	.493	-.098	21.47	41.75	24.34	-3.71
	25.111	2.648	3.644	-.567	3.639	.384	.528	-.082	23.48	7.61	34.72	-4.06
	48.528	4.985	3.593	-1.030	5.392	.554	.399	-.114	31.44	9.11	29.32	-5.23
	36.691	5.775	2.739	1.060	3.822	.602	.285	.110	24.75	10.24	16.51	4.90
	37.694	13.537	3.895	.481	3.677	1.321	.381	.047	25.42	23.99	23.48	2.22
Average.....	29.715	9.172	3.383	.342	4.286	1.323	.488	.049	24.73	20.84	27.80	2.13
Group average....	25.488	7.739	3.523	.223	3.490	1.060	.482	.031	21.12	17.76	27.36	1.42

TABLE 6.—Detailed balance record for each heifer in the mineral metabolism experiment—Continued

Heifer No.	Actual retention in grams daily of—				Retention per 100 pounds live weight in grams daily of—				Retention as percentage of intake of—			
	N	Ca	P	Mg	N	Ca	P	Mg	N	Ca	P	Mg
126.....	15.432	4.586	2.896	-0.620	4.978	1.479	0.934	-200	21.93	26.75	29.07	-8.53
	6.765	2.278	3.857	-220	1.961	.654	1.118	-064	8.42	11.30	33.97	-3.80
	12.477	3.812	4.281	.202	2.936	.897	1.007	.048	15.53	19.08	37.71	2.64
	14.458	3.967	-3.630	-2.531	3.109	.853	-.781	-.544	17.21	26.63	-24.01	-22.78
	9.446	1.754	-.883	-3.288	1.782	.331	-.167	-.622	11.16	11.55	-7.09	-27.56
	5.709	3.700	.562	-3.869	1.029	.677	-.101	-.697	6.02	18.95	4.34	-27.69
	21.365	4.794	2.627	.376	2.680	.669	.366	.052	19.40	23.02	17.47	3.30
	13.957	4.700	.559	-.579	1.756	.599	.070	-.073	12.12	20.56	3.55	-4.56
	19.669	2.363	1.620	-.184	2.126	.255	-.175	-.020	17.18	11.15	10.44	-1.38
	25.111	2.648	1.656	-.567	2.462	.260	.162	-.056	16.96	18.84	9.56	1.57
Average.....	14.439	3.470	1.355	-1.129	2.372	.570	.223	-.185	15.03	17.76	10.24	-8.88
128.....	14.085	4.748	2.904	-1.447	4.544	1.532	.956	-.467	20.02	27.69	29.76	-17.86
	11.438	5.270	3.033	.318	3.364	1.550	1.480	.117	14.23	26.37	44.33	5.20
	5.618	3.747	2.372	-1.670	1.387	.925	.586	-.412	6.99	18.75	20.89	-7.51
	17.563	3.434	-3.016	-3.402	3.444	.673	-.591	-.667	20.25	21.29	-20.28	-27.52
	20.155	2.572	1.080	-1.694	3.658	.472	.198	-.366	23.11	15.73	9.06	-18.09
	17.766	7.821	2.800	-2.233	2.889	1.272	.455	-.363	18.25	37.24	21.06	-17.59
	17.731	5.406	3.212	-.135	2.588	.789	.469	-.020	17.41	23.97	23.19	-1.22
	26.835	4.122	2.653	.430	3.388	.520	.335	.054	23.99	18.08	17.04	3.63
	18.449	4.396	-.304	.453	2.133	.508	-.035	.052	15.78	17.49	-1.84	3.61
	17.545	3.939	.985	-.235	1.772	.398	.099	-.024	13.91	16.97	5.68	-2.85
	26.132	.869	4.389	-1.495	2.539	.087	.423	-.145	16.25	3.77	17.65	-8.60
Average.....	17.776	4.214	2.013	-1.030	2.728	.654	.312	-.160	17.26	20.32	14.15	-8.05
138.....	14.589	5.663	2.878	1.330	4.559	1.770	.899	.416	19.38	37.58	30.03	16.92
	26.505	5.409	3.402	1.374	6.796	1.387	.872	.352	30.96	34.13	31.11	15.74
	22.863	4.716	2.738	-.162	5.081	1.048	.608	-.036	26.71	29.76	25.04	-1.82
	21.954	2.450	1.511	.247	3.326	.371	.220	.037	22.76	12.69	11.49	2.14
	38.282	2.155	5.994	-.815	5.004	.282	.784	-.107	28.05	9.84	28.35	-5.63
	8.003	2.158	2.869	-.533	1.026	.277	.368	-.068	5.89	9.22	13.52	3.79
	18.727	5.584	4.115	.670	2.190	.653	.481	-.078	13.78	23.86	19.40	-4.72
	23.623	5.492	1.140	-.183	2.305	.536	.111	-.018	19.15	17.42	6.25	-1.18
Average.....	21.818	4.203	3.081	.074	3.328	.641	.470	.011	19.96	20.22	19.60	.64
141.....	20.434	4.944	2.221	1.510	5.923	1.433	.644	.438	27.14	32.81	23.17	19.21
	25.275	6.045	2.302	.379	6.481	1.550	.560	.097	30.72	38.60	21.96	4.49
	25.083	7.304	1.415	.788	5.283	1.538	.298	.166	28.43	45.49	12.45	8.81
	20.217	3.371	.256	-.329	3.087	.515	.039	-.050	20.96	17.47	1.95	-2.77
	18.714	7.247	4.085	-.689	2.446	.947	.534	-.090	14.58	36.13	20.58	5.10
	10.013	3.825	2.649	-1.158	1.221	.466	.323	-.141	7.45	16.35	12.66	-7.96
	30.743	8.281	3.515	1.271	2.956	.796	.338	.122	24.92	26.27	19.29	8.30
	30.190	9.005	2.898	2.619	2.648	.790	.254	.230	24.47	28.57	15.90	17.10
Average.....	22.585	6.253	2.418	.519	3.209	.888	.344	.078	21.21	28.99	15.87	4.69
Group average.....	18.728	4.451	2.153	-.477	2.881	.685	.331	-.073	18.20	21.60	14.83	-3.18

In discussing the results as summarized in Table 7 it has been thought best to deal first with the general averages for each group and then to proceed to a more detailed examination of the results for the different ages.

The nitrogen balances were positive in all cases. The high-calcium group with a nitrogen intake per unit of weight averaging 4.4 per cent higher than that of the low-calcium group (the reason for which has already been explained, see p. 886), stored on an average 21 per cent more nitrogen per unit of weight than did the low-calcium group. The conclusion which might be reached as to the reason for this is that the protein of the alfalfa is superior for growth purposes to that furnished by ordinary hay supplemented with grain. Other variables enter in, however, such as possible differences in the value of beet pulp and apple pomace, or a possibly favorable effect of the high-calcium intake on protein storage, so that caution should be used in interpreting the result.



TABLE 7.—*Summary of balances by ages in the mineral metabolism experiment, March, 1927, to December, 1929*

## HIGH-CALCIUM GROUP

Age and number of trials averaged	Actual intake in grams daily of—				Actual retention in grams daily of—				Intake per 100 pounds live weight in grams daily of—				Retention per 100 pounds live weight in grams daily of—				Retention as percentage of intake				Ratio of Ca to P <sup>a</sup>				
	N		P		Ca		Mg		N		Ca		P		Mg		N		Ca			P		Mg	
Under 1 year (average of 13 trials)-----	89.34	35.04	10.13	11.00	17.03	0.52	4.14	0.81	21.50	8.43	2.44	2.79	4.31	2.29	1.00	0.19	20.07	27.17	40.87	6.98	3.51				
																					2.31				
	122.92	43.15	12.57	15.78	26.72	7.30	3.20	.22	15.76	5.53	1.61	2.02	3.43	.94	.41	.03	21.74	16.91	25.46	1.38	3.41				
to 2 years (average of 17 trials)-----																					2.31				
	161.84	56.73	17.42	21.43	34.08	7.11	3.23	-.80	14.82	5.19	1.60	1.96	3.12	.65	.30	-.07	21.06	12.53	18.54	Nil.	3.31				
Over 2 years (average of 9 trials)-----																					2.21				
Grand average (39 trials)-----	120.71	43.59	12.88	15.69	25.49	7.74	3.52	.22	16.53	5.97	1.76	2.15	3.49	1.06	.48	.03	21.10	17.80	27.40	1.46	3.41				
																					2.21				

## LOW-CALCIUM GROUP

Under 1 year (average of 13 trials)-----	80.09	17.22	10.78	7.95	16.78	4.76	2.55	-.12	20.76	4.46	2.79	2.06	4.35	1.24	0.66	-.03	20.95	27.64	23.63	Nil.	1.61
1 to 2 years (average of 17 trials)-----	109.88	20.15	15.74	11.51	18.93	4.13	1.89	-1.02	15.75	2.89	2.26	1.65	2.71	.59	.27	-.15	17.23	20.50	12.01	Nil.	1.91
Over 2 years (average of 7 trials)-----	128.25	26.57	18.51	14.88	24.72	4.66	2.31	.18	12.52	2.59	1.81	1.45	2.41	.46	.23	.02	10.27	17.54	12.48	1.21	2.01
Grand average (37 trials)-----	102.89	20.61	14.52	10.90	18.73	4.45	2.15	-.48	15.83	3.17	2.23	1.68	2.88	.69	.33	-.07	18.20	21.00	14.80	Nil.	2.11

<sup>a</sup> The first set of figures given in each case represents intake; the second, retention.

The calcium balances were positive in all cases. With a calcium intake per unit of weight averaging 88.3 per cent higher than that of the low-calcium group, the high-calcium group stored on an average 53.6 per cent more calcium per unit of weight. Although this group actually stored more calcium than the low-calcium group the percentage retention of that element did not average as high, 17.8 per cent as contrasted with 21.6 per cent.

The phosphorus balances were positive in 72 of the 76 trials. The four negative balances were confined to two heifers, both in the low-calcium group. With a phosphorus intake per unit of weight averaging 21.1 per cent less than that for the low-calcium group, the high-calcium group stored on an average 45.5 per cent more phosphorus per unit of weight, the percentage retention of this element being nearly double that in the low-calcium group, 27.4 per cent as against 14.8 per cent. This finding is perhaps the most interesting one in the whole investigation and its possible significance should not be lost sight of. It will be noted from Table 6 that the ratio of calcium to phosphorus retained is almost the same in both groups, slightly more than 2:1, while the ratio of calcium to phosphorus fed is about two and one-half times as great in the high-calcium group as in the low-calcium group. It would seem from this that the retention ratio is somewhere nearly constant at 2 to 1 irrespective of the ratio in the feed, and it follows in turn that a lowered calcium intake will pull down the phosphorus retention, irrespective of the level of phosphorus fed, to a point where the retention ratio is about 2:1. It would seem, therefore, that a high-calcium content in the ration, even if much above the requirements for that element itself, may be necessary in order to insure adequate storage of phosphorus by growing heifers.<sup>5</sup>

The magnesium balances present a puzzling situation. Forty-eight of the 76 balances determined were negative. These were evenly divided between the two groups, 24 in each, but the individual negative balances averaged somewhat larger in the low-calcium group so that the average result was a slight positive balance in the high-calcium group and a slight negative balance in the low-calcium group.

Similar findings have been noted by other investigators in mineral balance experiments with human subjects and also with cattle, but an entirely satisfactory explanation has never been put forward. Forbes has commented on the situation in several of his papers on mineral metabolism. In one place<sup>6</sup> he remarks that "the interpretation of a negative balance, then, may be a matter of some uncertainty," and in another<sup>7</sup> that—

balances of mineral nutrients such as magnesium, which are utilized in small proportions of the usual intake, are of much less certain significance, and cannot be closely interpreted—especially in view of the great complication of factors, other than the quantity present, which enter into the determination of the balance of intake to output

Regarding interdependence in the metabolism of this element and that of calcium, referred to earlier in this paper, it appears that where

<sup>5</sup> Since the above was written some unpublished results of other investigators have been brought to the writers' attention which show an apparent independence of calcium and phosphorus in metabolism. These results, however, have been obtained with milking cows. It may be that the situation changes with the advent of lactation.

<sup>6</sup> FORBES, E. B., HUNT, C. H., SCHULTZ, J. A., WINTER, A. B., and REMLER, R. F. THE MINERAL METABOLISM OF THE MILCH COW. Ohio Agr. Expt. Sta. Bul. 363, p. 38.

<sup>7</sup> FORBES, E. B., FRENCH, R. B., and LETONOFF, T. V. THE MINERAL METABOLISM OF THE BEEF STEER. Jour. Nutrition 1: 208, 1929.

the calcium intake was high there was quite a definite and direct inter-relationship in the metabolism of these two elements. With a low calcium intake the relationship was not nearly so marked.

Turning to a more detailed study of the results as summarized in Table 6 under the subdivisions of each group according to age, the following points are noted:

(1) As would naturally be expected, intake per unit of weight of all the elements under consideration decreased with increasing age. Most of this decrease took place in the second year as compared with the first, there being in general little further decrease in the third year. The rate of decrease in intake was roughly parallel for both groups and for all elements.

(2) With the single exception of magnesium in the third year, retention per unit of weight also decreased in all cases with increasing

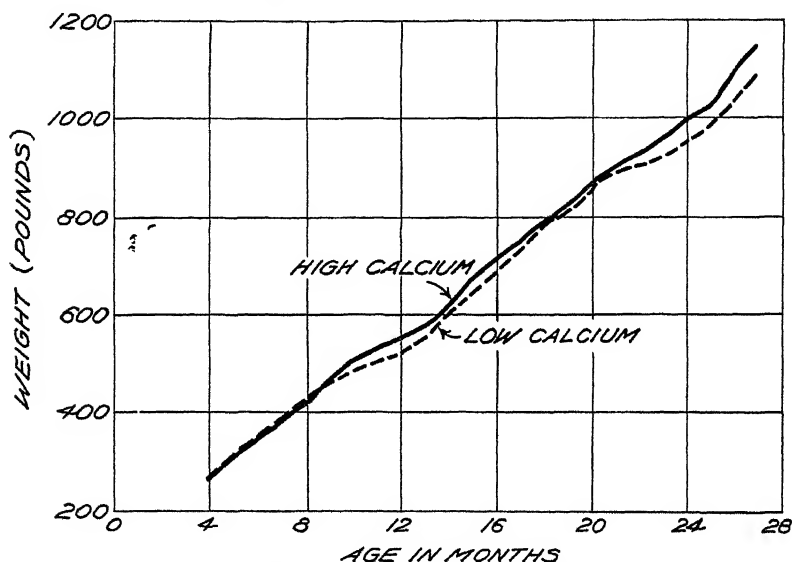


FIGURE 2.—Composite curves representing average weights of heifers in the two groups. The solid line represents the high-calcium and the broken line the low-calcium group

age, and, as with intake, most of the decrease was between the first and second years. Rate of decrease in retention was much higher with calcium and phosphorus than with nitrogen, indicating that the greatest demand for these elements is during the first year of life. Evidently skeletal growth proceeds more rapidly toward completion than does the growth of other body tissues, which contain relatively large amounts of nitrogen and relatively little calcium and phosphorus in their make-up.

Comparing the groups, it is noted that rate of decrease in retention per unit of weight was about the same for phosphorus in both groups; that it was less rapid for nitrogen in the high-calcium group, and less rapid for calcium in the low-calcium group.

(3) Retention of the elements expressed as percentage of intake did not show a uniform decrease with advancing age. Although in general the tendency was to decrease from start to finish, there are several cases of increased percentage retention, notably from the second to the third year, and one case (nitrogen in the high-calcium

group) where percentage retention was higher in the third year than in the first. It is interesting to note that all cases of percentage increase except the one just mentioned were in the low-calcium group, although curiously enough calcium itself was not involved.

In general it can be stated that efficiency in the use of the elements, as measured by relative retention, was lowered with advancing age. This was especially true of the element calcium and of the high-calcium group. A tendency toward an increased efficiency in the third year is probably a reflection of the demands of pregnancy.

The results as a whole point to accumulation of a considerable reserve of all elements by the high-calcium group during the first year of life, with a resultant lowering of relative retention later on. The

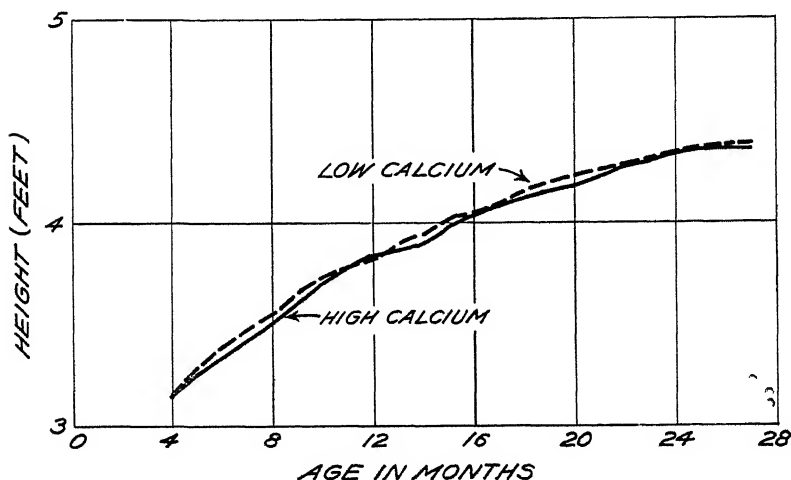


FIGURE 3.—Composite curves representing average heights of heifers in the two groups taken at withers. The solid line represents the high-calcium and the broken line the low-calcium group

low-calcium group, not having accumulated such a large reserve in the earlier stages, must have felt the pinch later on and made an attempt to even matters up, as evidenced by the tendency in this group toward increased efficiency in the third year.

(4) The establishment of approximately a 2 to 1 ratio between amounts of calcium and phosphorus retained irrespective of the ratio in the intake, was very uniform in both groups and at all ages. The significance of this finding has been dealt with rather fully under the discussion of average results (p. 891). The striking uniformity at all ages is additional confirmation of the conclusions there drawn.

#### GROWTH RECORDS OF THE ANIMALS

All the heifers were weighed and measured twice a month until they were a year old. After that the records were taken once a month. The measurements recorded were height at withers and heart girth. Photographs were also taken from time to time. A graphic summary of the growth records is shown in Figures 2, 3, and 4.

These charts need little comment. The lower weight of the low-calcium group from the twenty-first month onwards is due largely to the fact that on an average they were bred about one month late

than the other group, and the difference is due to the earlier stage of gestation. It will be noted that their weights at any given age after 21 months are almost identical with those of the other group one month earlier. Height and girth remained about the same in both groups all through the experiment. Photographs taken when the heifers were 2 years old appear in Figures 5 and 6. In addition a record of the weight and condition at birth of each heifer's first calf appears in Table 8. Two calves in each group were sired by one bull and the other two in each group by his son, so that influence of sire was evenly distributed.

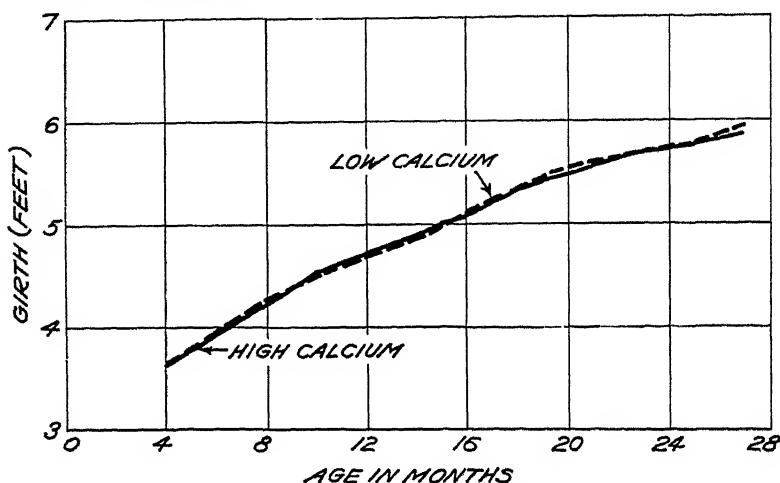


FIGURE 4.—Composite curves representing the average heart girths of heifers in the two groups. The solid line represents the high-calcium and the broken line the low-calcium group

TABLE 8.—Weight and condition at birth of calves born to heifers in both calcium groups

Calf of heifer No.	High calcium		Calf of heifer No.	Low calcium	
	Weight at birth	Condition at birth		Weight at birth	Condition at birth
	Pounds			Pounds	
121.....	85	Excellent.	126.....	78	Good.
131.....	80	Good.	128.....	90	Do.
137.....	75	Do.	138.....	75	Do.
139.....	85	Do.	141.....	97	Excellent.
Average.....	81		Average.....	85	

It would seem from a study of the growth records and from close observation of the animals that thus far at least the considerably lower mineral storage by the low-calcium group has had no ill effects. It would be interesting to know what the heifers in the high-calcium group have done with the extra calcium and phosphorus they have stored. Presumably it has been stored as a reserve in the bones. Time alone will tell whether this large reserve is necessary in order to tide the animals over periods of strain and possible mineral shortage during heavy lactation. If it is found that the low-calcium group continues to produce and reproduce normally then it would seem reasonable to conclude that heifers can make satisfactory growth on

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